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The effect of liquid "rumen protected" lysine supplementation on the productivity of lactating Holstein cows

by

Richardt Venter

Submitted in partial fulfilment of the requirements for the degree

M.Sc (Agric) Animal Nutrition

Department Animal and Wildlife Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

September 2008

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DECLARATION

I declare that this dissertation for the degree of M.Sc (Agric) Animal Nutrition at the University of Pretoria, has not been submitted by me for a degree at any other University.

R. Venter

Pretoria

September 2008

ACKNOWLEDGEMENTS

I would like to thank all my supporters. Here I am at last! To list everybody over the years will take too long; therefore I would like to point out the most significant contributors to the success and completion of this study:

The work itself and the achievement of this degree are dedicated completely to my grandfather; Pieter J. Cronje (1920 – 1957), who always wanted to become an agriculturalist, but never had the chance.

Furthermore, for the support and interest from my parents and family, especially my father who kept up with the data processing for me during the trial and my mother who always believed in whatever I believed in. Also, to my sister who helped with spelling, grammar and references; and my brother the engineer for being analytical and being there.

The Dairy Unit of the Agricultural Research Council in Irene, Pretoria, for supplying suitable animals to add to the numbers for this trial, enabling me to shorten the experimental period.

Last, but certainly not the least, I would like to thank my wife, Theresa, who serves as my inspiration in life through everything she does and the way she does it; to whom I give full credit for me finalizing this thesis.

SUMMARY

The effect of liquid rumen protected lysine supplementation on the productivity of lactating Holstein cows

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Thirty high-producing multiparous Holstein cows were used in a completely randomized block design to compare a lysine deficient total mixed ration, which was sufficient in methionine, to the same diet supplemented with a rumen protected lysine product. The CPM-Dairy prediction model was used to estimate the nutrient requirements and adequacy or deficiency of amino acids. During the 21-day prepartum transition period, cows were fed 4 kg (dry basis) of the lysine deficient diet plus *Eragrostis curvula* hay *ad lib*. After calving, cows were fed the lysine deficient diet for the first three weeks and

were then blocked according to the average production from day 19-21. Fifteen cows were allocated to each treatment and blocked into 15 groups of two each. Data on production parameters were analyzed for all cows and also separately for cows in the 10 highest production blocks. The experimental period was from day 22 to 120 postpartum.

Lysine supplementation resulted in an optimal dietary lysine : methionine ratio in metabolisable protein of 7.2 : 2.4. Lysine supplementation did not affect dry matter intake, milk production, milk fat percentage, milk protein percentage, milk urea nitrogen, body weight or body condition score; but decreased the non-casein nitrogen and whey content of milk. Furthermore, milk casein, which is the milk nitrogen fraction most sensitive towards increased duodenal supply of lysine and methionine, was not affected.

The rumen protected lysine product evaluated did not improve cow productivity, probably because the product was either unprotected from rumen degradation, or overprotected to the extent that the lysine was not available for absorption in the small intestine; or absorbed but could not be metabolised.

LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fibre
AP	Absorbable protein
BCS	Body condition score
BHT	Butylated hydroxyl-toluene
BW	Body weight
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
CPM-Dairy	Cornell Penn Miner Dairy ration formulation program
Cys	Cysteine
DIM	Days in milk
DIP	Degradable intake crude protein
DM	Dry matter
DMI	Dry matter intake
DP	Dietary protein
EAA	Essential amino acid
ECM	Energy corrected milk
FCM	Fat corrected milk
FDA	U.S. Food and Drug Administration
GRAS	Generally Accepted as Safe
His	Histidine
HMB	Hydroxy-methyl butanoic acid
Ile	Isoleucine
IOFC	Income over feed cost
Lys	Lysine
MAA	Metabolisable amino acid
MCP	Microbial crude protein
Met	Methionine

MHA	Methionine hydroxy analog
MP	Metabolisable protein
MUN	Milk urea nitrogen
N	Nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NFC	Non-fibre carbohydrate
NPN	Non-protein nitrogen
NRC	National Research Council
OM	Organic matter
RDP	Rumen-degradable protein
RP	Rumen protected
RPAA	Rumen protected amino acid
RR	Rulquin Ratio
RUP	Rumen-undegradable protein
S	Sulfur
SEM	Standard error of the mean
TMR	Total mixed ration
Val	Valine
VLDL	Very low density lipoprotein

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CHAPTER 1 - INTRODUCTION

There are two primary goals in dairy production: maximizing milk production and increasing production efficiency. Although energy constitutes the largest proportion of many dairy cattle diets, protein is by far the second largest component of the feed. Protein is of major significance to most nutritionists as it is usually the most expensive component of the diet. Furthermore, the efficiency of ruminants in converting dietary nitrogen into milk protein, is not particularly good, especially when compared to monogastrics. Diet formulation strategies to increase the efficiency of N utilization for milk protein production include increasing the amount of fermentable carbohydrate in the diet, reducing the amount of 'surplus' protein in the diet and improving the profile of amino acids (AA) in metabolisable protein (MP) (NRC, 2001).

Amino acid nutrition of dairy cows has received a lot of attention over the last decade, resulting in several nutritional models which allows for diet formulation on the basis of AA. It is assumed that, as for monogastric species, there is an optimum AA profile for each physiological state of the dairy cow. The Cornell Net Carbohydrate and Protein System (CNCPS) was developed out of the need for more accurate models to define rumen bacterial and whole animal requirements, to assess feed utilization and to predict production responses (Chalupa *et al.*, 2001). National Research Council (NRC, 2001) also developed a new protein model that incorporates AA in the sense that it predicts AA flow to the small intestine. The CPM-Dairy model (Chalupa & Sniffen, 2006) goes one

step further and uses a combined approach to evaluate and formulate diets: a modification of the classical NRC system and the CNCPS (Chalupa *et al.*, 2001). These models are probably the most widely tested and used today. Because of these developments more and more emphasis will be placed on AA formulation in future (Chalupa *et al.*, 2001).

Ruminants have the ability to synthesize all AA. However, it is important to understand that ruminants still require dietary AA since there is a limit in the synthesizing capacity of rumen microbes (Bailey, 2000). The question that research has been dealing with is what amount of which specific AA are needed to support higher and higher production in dairy cattle. Of the 22 AA, lysine (Lys) and then methionine (Met) are the first two AA that can limit production in dairy cattle on maize and soyabean based diets. Recent research has also indicated that histidine (His) is probably the first limiting AA on grass silage based diets (Bequette *et al.*, 2000; Schwab & Ordway, 2001). The concern has always been to supply the dairy cow with protein sources that contain adequate levels of rumen undegradable Lys and Met. The problem is further exacerbated by the fact that maize, which constitutes a major part of typical dairy diets, is sufficient in Met but deficient in Lys.

Recent research suggests that other AA may also limit milk protein production. For example, when His supply went from deficient to adequate, a milk protein response was observed in dairy goats (Bequette *et al.*, 2000). Glutamine was shown to be potentially limiting when free AA levels were monitored in plasma and muscle (Blum *et al.*, 1999). There are also reports indicating that supplementation with ruminally undegraded protein

(RUP) or rumen protected AA (RPAA) do not increase milk protein yield (Yang, 2002). Positive results from supplementing RPAA, therefore, are dependant on whether that AA was first limiting.

From a series of experiments Rulquin *et al.* (1995) concluded that Lys needs to be 7.3% of metabolisable AA (MAA) and Met needs to be 2.5% of MAA. In an excellent research summary, published in the NRC (2001), Schwab came to the same conclusion, namely that Lys and Met should constitute 7.2 and 2.2% of MAA respectively. These results now provide nutritionists with proper guidelines when formulating for AA. Diets formulated accordingly result in cows optimising milk protein production (Rulquin & Verite, 1993) and milk protein appears to be significantly reduced when diets provide less than 2.1% Met or 6.0% Lys, which are considered minimums. Responses of cows in terms of milk protein production when supplementing Met may even be negative if Lys is limiting. However, it is extremely difficult to reach the optimum concentrations of AA for milk protein synthesis by using only conventional feedstuffs. This is particularly the case for cows in early lactation when dry matter (DM) intake is relatively low and protein requirements are high (Rode & Kung, 1996). Feeding a diet containing more protein is not a satisfactory solution because the breakdown of dietary protein in the rumen is one of the most inefficient processes in ruminant nutrition. In typical dairy rations, only 25 to 35% of the feed protein reaches the small intestine for absorption. In an attempt to overcome this inefficiency, dietary protein sources that are considered to be good sources of RUP have been used. The only practical way to reach these levels and ratios of AA is dietary supplementation with RPAA so that any AA imbalances are corrected and overall

utilization of dietary protein is improved (Rode & Kung, 1996). However, one of the great challenges lies in the fact that various AA in rumen protected protein sources may still degrade in the rumen at various rates.

Up to recently, only Rumen Protected (RP) Met products were available commercially and many nutritionists are eagerly waiting to see the production results on a newly launched RPLys product form Balchem Corporation (52 Sunrise Park Road, New Hampton, NY, USA). But, in general, the following conclusions can be made from literature:

- Methionine and/or Lys are likely to be the AA that are first limiting in the small intestine.
- Lactating dairy cows frequently respond to supplementation with enhanced milk protein production.
- Amino acid requirements derived by the factorial method (calculated from product composition and metabolic transfers) are not far different from what is achieved by dose response studies.
- Studies are limited with respect to RPAA additions to diets where efforts have been made to achieve AA balance at the small intestine through the use of conventional feeds.

Amino acid nutrition and the role of RPAA continue to be an active field of research (Schwab *et al.*, 2004).

Responses to feeding individual AA to dairy cattle have not been consistent. Response differences probably occur based on the quantity and proportion of AA in the microbial and dietary protein digested and absorbed from the small intestine (Smith *et al.*, 2001). Responses are often greater when mixtures of AA, rather than individual AA, are administrated directly in the lower digestive system. Combinations of RPMet and RPLys have been shown to increase milk protein yield and concentration when supplemented to diets low in rumen degradable protein (RDP). Furthermore, it has been demonstrated that supplementation with RPMet and RPLys can play a role in alleviating the milk protein depression observed when supplementing fat to dairy diets (Smith *et al.*, 2001).

Chalupa *et al.* (1999) formulated AA enriched diets and increased Met/MP from 1.89 to 2.35% and Lys/MP from 6.38 to 7.45%. The ratio of Lys : Met in the enriched ration was 3.2 : 1. Milk production was increased by 5.1%, milk protein by 8% and milk protein yield by 18%. These results clearly demonstrate the potential application of AA rations and RPAA in diet formulation to fine-tune diets for optimum response in milk and milk protein yield.

There is an ongoing need to optimize protein and AA use in animal nutrition for various reasons. Excess N, due to poor formulation or overfeeding protein, is a burden to both the animal and environment. At the same time, there is pressure to reduce the use of animal by-product feeds, often resulting in the need to increase diet protein because of a less than desirable AA profile in plant protein, compared to animal and fish protein. On the other side of the equation, high producing dairy cows must utilize additional energy when

converting excess N and AA to urea for elimination, reducing the amount of energy available for productive purposes (Evans, 2004). Diets need to be formulated to reduce oxidation of AA by feeding the correct amounts of AA whenever possible and whenever economical. Another consideration for using available AA technology revolves around animal health. Amino acids are key components of proteins required for the production of enzymes, immunoglobins, some hormones, muscle and milk. Amino acids contribute to the formation of glucose; acting as a buffer when other precursors are in short supply. When the feed fails to supply sufficient AA, net catabolism of tissues occurs in order to supply AA for the most critical functions. Ensuring that the correct amounts of AA are available contributes to productive performance by supporting wellness (Evans, 2004).

The physical-chemical properties of Lys are such that most technologies are currently limited to the commercialisation of RPMet. Technologically, the approaches to protect free AA from ruminal degradation fall into one of three categories (NRC, 2001):

1. surface coating with a fatty acid/pH sensitive polymer mixture;
2. surface coating or matrices involving fat or fatty acids and minerals; and
3. liquid sources of Met hydroxy analog.

Recently, a new rumen protected Lys product, with rumen protection obtained by means of a chemical process, was developed. The purpose of this study was to evaluate this new product through a lactation study with Holstein cows, whereby a Lys deficient diet was supplemented with RPLys.

CHAPTER 2 - LITERATURE REVIEW

BALANCING DAIRY DIETS FOR AMINO ACIDS

2.1 Introduction

There may be several advantages to using rumen protected AA in ruminant diets. Firstly, small amounts of RPAA can substitute for a substantially greater amount of RUP. Secondly, by-product feeds low in Met and Lys could be better utilized knowing that RPAA could overcome AA limitations in these feeds. Thirdly, RPAA could be used to supplement cows in the dry period without creating the potential for downer cow syndrome that may occur with the feeding of high levels of protein. Fourth, feeding supplemental fat to lactating dairy cows increases the energy density of the diet but often results in decreased milk protein. Feeding RPAA has been shown to overcome this problem. Finally, N pollution of surface and ground water and environmental acidification from livestock are increasing problems in many areas of the world. Utilizing RPAA technology is “environmentally friendly” in that it improves the efficiency of protein utilization in ruminants.

RPAA are not feed additives to be fed at a single dosage rate irrespective of diet composition. They are feed ingredients and should be formulated into feed accordingly. RPMet are concentrated sources of metabolisable Met and should be offered along with

conventional feed ingredients available to nutritionists for “least cost” diet formulation to meet target metabolisable Lys and Met levels (Sloan, 2005).

Many factors have to be considered before RPLys and RPMet are fed. These include:

1. predicted contributions of Lys and Met to other AA in duodenal digesta;
2. level of management;
3. price received for milk protein;
4. cost of RUP feed ingredients; and
5. efficacy and cost of RPLys and RPMet supplements.

As with many new technologies, evidence suggests that the best-managed herds will benefit the most. Moreover, it is in these herds that improvements in production can be most easily measured (Schwab, 1995). The cost of RPLys (if available) and RPMet supplements, relative to anticipated benefits, is the deciding factor determining the extent of their use.

2.2 Amino Acid requirement models for dairy cows

Since the latest NRC has been published in 2001, there has been renewed interest to formulate dairy diets to meet conventional "protein" requirements and also to balance the diet for at least the first two limiting AA for ruminants, Met and Lys. Animals do not actually have a requirement for protein. Instead, they require the specific AA that are the building blocks making up proteins. Therefore, the limiting factor in most dairy diets is the first or most limiting AA. To advance research on AA requirements and to allow for improved diet formulation as new information on AA requirements becomes available, the protein model of NRC (2001) was extended to one that would most accurately predict the profile and flows of essential AA (EAA) to the small intestine.

Feed proteins are metabolised by rumen microbes or absorbed in the intestines. Absorbed AA of feed or of microbial origin are used for protein synthesis of body proteins, enzymes, milk etc. A substantial part of the glucogenic AA are used for the production of lactose, thus supporting a high milk production. The possibility for the mammary gland to utilise glucose for lactose production is limited and absorbed AA have therefore an important positive relation to milk yield. In practice, this easily results in overfeeding of proteins. Excess protein results in energy costs when excess N is converted into urea and excreted in the urine. Moreover, overfeeding may cause fertility problems and sometimes also a very loose consistency of the manure, causing various health problems (Gustafsson *et al.,* 2000). Nutritional models such as CNCPS and CPM-Dairy have contributed

greatly to nutritionists now being able to avoid many of the abovementioned problems by fine-tuning diet formulations and formulating for the correct amount and balance of AA needed. Some estimates suggest that more than 90% of diets for high-producing dairy cows are inadequate in energy and certain AA, causing at least a 4 to 8% shortfall in the amount of milk protein the animals could otherwise produce (Yang, 2002).

The NRC 2001 database doesn't support the AA content of different protein fractions in all feedstuffs, but shows the AA content of the total feedstuff. This is due to the scarcity of specific AA data for anything other than the total feedstuff (Sniffen, 2002). The AA database in CPM-Dairy is based largely on the research done by MacGregor and Mantysaari from 1978 onwards. The CNCPS/CPM-Dairy models predict microbial yield from two equations that incorporate both microbial maintenance requirements and microbial growth efficiency. The two equations are based on fermenting fibre, starch, soluble fibre and sugars. The advantage of this approach is that we increase the sensitivity of prediction of yield, by predicting the yield for all the substrates and being able to change the microbial efficiency, if needed (Sniffen, 2002).

The protein requirements of lactating dairy cows have been researched for many years and continue to be refined. In earlier NRC recommendations (NRC, 1971; 1978), dietary requirements were expressed as Crude Protein (CP) and metabolic requirements as digestible protein. In NRC (1989), dietary requirements were expressed as CP or degraded intake CP (DIP) and undegradable intake CP (UIP) and metabolic requirements as absorbed protein (AP). Mean values of ruminal degradability for common feeds,

derived from *in vivo* and *in situ* studies using sheep and cattle, were reported. A fixed intestinal digestibility of 80% for RUP and microbial true protein was used for predicting passage of absorbed protein. In NRC (2001) however, dietary requirements are expressed as rumen degradable CP (RDP) and rumen undegradable CP (RUP) and metabolic requirements are expressed as metabolisable protein (MP).

Other major changes in NRC 2001, in comparison to NRC 1989, are:

1. microbial CP flows are predicted from intake of total digestible organic matter (OM) instead of Net energy (NE) of intake;
2. a mechanistic system is used for predicting the RDP and RUP content of feeds that recognizes that the proportional content of these two fractions is not constant and is affected by DM intake (DMI) and diet composition;
3. variable estimates of digestibility are assigned to the RUP fraction of each feed; and
4. flows of digestible EAA and their content in MP are predicted.

Amino acid requirements were not established, but dose-response curves that relate measured milk protein content and yield responses to changes of predicted percentages of Lys and Met in MP are provided (Schwab *et al.*, 2004).

When fed to ruminants, proteins and AA are first subject to microbial degradation in the rumen, making it difficult to predict the quality and quantity of AA that are absorbed by the animal. In ruminants, absorbed AA originates from microbial protein synthesis in the rumen and from dietary AA sources that bypass the rumen undegraded. Although it is

difficult to predict the theoretical requirement for pre-formed protein or AA in dairy diets, we know that production of microbial protein alone is insufficient to supply adequate amounts of AA for optimal production. Diets for dairy cows can now be formulated to ensure a more efficient use of dietary protein while optimizing milk yield and solids, particularly milk protein. This gives the producer the opportunity to improve Income over Feed Cost (IOFC) through producing more milk with a higher value per litre for a small increase in feed cost. However, the other secondary benefits, such as the milk protein responses, may in some cases be contributing as much, if not more, to the profitability of the dairy farmer.

Absorbed AA and not protein per se, are the required nutrients. Used principally as building blocks for synthesis of proteins, absorbed AA are vital to the maintenance, growth, reproduction and lactation of dairy cattle. It is also understood from poultry (NRC, 1994) and swine (NRC, 1998) research that an ideal profile of absorbed EAA exists for different functions such as maintenance, growth and lactation. While these ideal profiles remain to be established for dairy cattle, it is known that feeds vary in AA composition and that the ingredient composition of the diet affects the AA composition of duodenal protein. Two factors account for most of the variation in AA profiles of duodenal protein. These are the proportional contribution that RUP makes to total protein passage and the AA composition of that RUP (Schwab & Ordway, 2001).

AA can be added directly to the diets of monogastric animals to overcome nutritional deficiencies. However, in ruminants free-form AA are rapidly degraded by rumen

bacteria and are of little or no practical benefit in alleviating AA deficiencies. Rumen-protected AA must be either modified or protected in some way so that they are not susceptible to rumen degradation. Several methods have been used to develop commercial RPAA products. A potential problem is that AA can be over-protected (Rode & Kung, 1996). Complexes that are extremely inert in the rumen can be indigestible in the small intestine as well. Therefore, a trade-off exists between good ruminal protection and bioavailability (Rode & Kung, 1996).

There are two approaches to formulate for AA for the dairy animal. One is the factorial approach used in the CNCPS and developed by O'Connor *et al.* (1993). This approach calculates AA requirements using net amount of protein synthesized for each function of the cow, e.g.: maintenance, growth, gestation, mammary repletion and milk protein production. Then, the grams of tissue/milk protein to be synthesised times the AA composition corrected with an efficiency factor for utilisation for each AA are calculated; to give the metabolisable or absorbed AA needed (Sniffen, 2002). Each of these steps has variance associated with it and this system is therefore particularly sensitive to the efficiency factors for the different physiological functions (Overton *et al.*, 1996). Although this is fairly accurate, it does not take into account the fact that AA are taken up mainly through active transport sites and if we get an excess for any AA it can have a negative impact on the uptake of other AA.

The second approach is to feed AA in a profile that will optimize uptake of AA (Sniffen, 2002). The swine NRC (1998) guidelines outline this approach, which express the AA as

a percentage of Lys. If all of the other AA are in the correct ratio with Lys they can then optimize performance. This ideal protein system is based upon the concept that AA will be used for productive function in a characteristic proportion to each other; therefore, balancing on an ideal protein basis will maximise the efficiency of N use in the cow (Schwab *et al.*, 1993; Rulquin *et al.*, 1995). This Schwab system expresses Met and Lys as a percentage of EAA flow to the small intestine and the Rulquin system expresses Met and Lys as a percentage of MP flow to the small intestine (Schwab, 1996). Requirements in both systems were determined by either infusing or feeding increasing amounts of the AA of interest until the response variable peaked, which was usually milk protein yield.

Based on the traditional factorial approach of estimating a requirement for maintenance, growth, lactation, pregnancy etc., individual AA requirements can't be determined accurately. The current accepted approach is the indirect response curve method first proposed by Rulquin and Verite (1993). This methodology was used in NRC 2001. The advantage of this method is that the determination of supplies and requirements of individual AA are interdependent. Requirements are estimated as a dose response function using the approach established to estimate MAA supplies. Requirements are therefore dependent on and can vary between different formulation systems. There can, however, be only one requirement for an animal at a specific physiological status and level of production, therefore a more correct terminology to use would be target formulation levels or recommendations; rather than requirements. The factorial method requires knowledge of the AA content of products and the efficiency of AA use. Amino

acid content of milk and tissues can be estimated reliably, but an estimate of the efficiency of AA use is difficult and variable (Sniffen & Chalupa, 2004).

Dose response curves used to establish the levels of Lys and Met as a percentage of MAA needed to optimize milk protein concentration are illustrated in NRC (2001). Low concentrations of Met in MP limited responses of Lys in MP and *visa versa*. Optimums were established at 2.5% for Met and 7.3% for Lys as a percentage of MAA (Rulquin *et al.,* 1995). Similarly, optimum ratios calculated by the Sniffen *et al.* (2001) multiple regression approach were 2.2% Met in MP and 7.4% Lys in MP. Feeding a ratio much higher than this results in a net waste of Lys (Schwab & Ordway, 2004). As mentioned earlier, these levels cannot be achieved in practice using primarily maize grain based diets. It will be difficult to achieve Lys levels higher than 6.7% of MAA. Thus, practical target formulation levels of 6.6% Lys and 2.2% Met as a percentage of MAA have been suggested with respect to the NRC 2001 formulation approach.

It is important to note that Met levels will depend on the level of Lys that can be achieved. The first step is to maximise Lys as a percentage of MAA, then balance the Met to keep a 3.0 : 1 ratio to maximise efficiency of utilization of MAA and prevent the unnecessary overfeeding of Met. These target formulation levels will be a little different depending on the formulation system employed. For example, using CNCPS or CPM-Dairy, target formulation levels are suggested at 6.82% and 2.19% of MAA (Sloan *et al.*, 2000). This is because when the same diet is evaluated through both models, in general, CNCPS predicts higher levels of Lys in MAA compared to NRC. Target Lys formulation

levels have to be adjusted accordingly and the optimum Lys and Met ratio will also change. A ratio of 3.12 : 1 is suggested as the optimum to use with CNCPS and CPM-Dairy (Schwab & Ordway, 2004; Sloan, 2005).

When dairy diets are balanced for Met and Lys according to the Rulquin Ratio (RR), the response has generally been a significant improvement in milk true protein. Practical application, however, can be complicated (Sniffen, 2002). It is relatively easy to formulate for the correct level of Met using commercially available RPMet sources; a commercial RPLys, however, is not available. It is important to realise that performance can be reduced when the Lys : Met ratio is less than 3.0 : 1.

2.3 Amino Acid supply

In dairy cattle nutrition, similarly to monogastric nutrition, the AA that are most likely to limit protein synthesis should be identified. If the diet can then be enriched with these AA, milk protein synthesis and the efficiency of utilization of all absorbed AA will be maximized. Methionine is nearly always first limiting, with Lys secondary and His thirdly. The extent and sequence of their limitation appears to be affected primarily by the amount of RUP in the diet and its AA composition (Schwab & Ordway, 2001). Lys limitation can vary from a co-limitation with Met to situations where Met supplies need to be increased by nearly 20% before Lys becomes a limiting factor (Sloan, 2005). Lys, however, is inconsistent: although it is often first- or second-limiting on most maize-based diets, at the same time it is almost always taken up in excess by the udder and most of it is oxidized (Mabjeesh *et al.*, 2000).

Where maize is the only grain in the diet and some maize by-products or brewers grain are fed, both Lys and Met levels in MP will need to be improved to elicit a response. It is still a major challenge even to achieve 90% of the estimated requirements for Lys and Met with the ingredients we have available currently (Sloan, 2005). Methionine is first limiting for growth and milk protein production when dairy cattle were fed high forage or soyabean hull-based diets and intake of RUP was low. Methionine has also been identified as first limiting for growing cattle and lactating cows that were fed a variety of diets in which most of the supplemental RUP was provided by soyabean protein,

especially heated soybeans, animal-derived proteins, or a combination of the two (Williams *et al.,* 1999; Schwab & Ordway, 2001; Bequette & Nelson, 2006). In contrast, Lys is first limiting for growth and milk protein synthesis when maize and feeds of maize origin provided most or all of the RUP in the diet (NRC, 2001). Relative to concentrations in microbial protein, feeds of maize origin are low in Lys and similar in Met, whereas soyabean products and most animal-derived proteins are similar in Lys and low in Met. Methionine and Lys have also both been identified as co-limiting AA for milk protein synthesis when cows were fed maize silage-based diets with little or no protein supplementation. Histidine has been identified as first limiting for milk protein production when dairy cows were fed grass silage-cereal (barley and oats) based diets.

Concentrations of Met and Lys in most feed proteins are lower than in microbial protein (Bequette *et al.*, 2000; Schwab & Ordway, 2001). Thus most feed proteins are not complementary to microbial protein and instead, when they are fed, will accentuate rather than eliminate deficiencies of Met and Lys in MP. This also appears to be why Met and Lys becomes more limiting (relative to the other EAA) with increasing intakes of complementary sources of RUP (Schwab & Ordway, 2001). Lys is more vulnerable to heat processing than the other EAA. Over-heating decreases Lys concentrations and can decrease the availability of the remaining Lys.

It may be expected that Lys and Met are the first two limiting EAA for growth and milk production, due to the following reasons (Schwab, 1995; NRC, 2001):

1. Methionine was already identified as first limiting and Lys as second limiting for N retention of growing cattle dating back to 1978 (Richardson & Hatfield) and this was confirmed many times by different researchers worldwide.
2. Methionine and Lys are first and second limiting in ruminally synthesized microbial protein for growing ruminants.
3. Lysine and Met are the first two limiting AA for lactating dairy cows fed conventional forages and energy feeds without protein supplementation.
4. Most protein supplements have lower amounts of Lys and Met, particularly of Lys, than bacterial protein.
5. The contribution of Lys to total EAA in the RUP fraction of feed proteins is often slightly lower than in the same feeds before exposure to ruminal fermentation.
6. Most feedstuffs have lower amounts of Lys and Met in total EAA than in Microbial CP (MCP).
7. Contributions of Lys and Met to total EAA in body lean tissue and milk are similar.
8. Lys and cysteine (Cys), are more susceptible to heat processing and may have lower intestinal digestibilities than other EAA in RUP (Cys can be synthesized in the body from Met).

The principle sources of AA are grouped under MP, which is the true protein that is digested postruminally. It consists of microbial protein, RUP and commercial RPAA products (NRC 2001).

2.3.1 Microbial Amino Acids

Ruminally synthesized microbial protein can supply up to 50% or more of the absorbable AA in diets (Schwab, 1995). Microbial protein is the cellular protein of the bacteria, protozoa and fungi that multiply in the rumen and pass along to the small intestine with unfermented feed. Over 200 species of bacteria, more than 100 species of protozoa and at least 15 species of fungi have been isolated from rumen contents (Kamra, 2005). Bacteria however, provide the majority of the total microbial protein leaving the rumen. Microbial protein is considered to be a constant and high quality source of absorbable AA (Rode & Kung, 1996). It has an apparent intestinal digestibility of about 85%, an EAA pattern that is similar to that of lean body tissue and milk, and is assumed to be fairly constant and not influenced significantly by changes in diet. Although similar in EAA composition to lean body tissue and milk, ruminally synthesized microbial protein still does not possess a perfect EAA balance (Schwab, 1995).

Rumen protozoa are higher in Lys and lower in Met than bacteria, but the presence of protozoa does not affect the AA profile of protein flowing from the rumen. This indicates that protozoa contribute little to the quality of protein flowing from the rumen. While it is a well balanced source of protein, production of microbial protein is limited by the fermentability of the diet and the amount of RDP in the diet. Therefore, microbial protein alone is insufficient to meet the requirements for high levels of milk production (Rode & Kung, 1996).

The assumption that the EAA pattern of microbial protein is fairly constant is based on three observations (Schwab, 1995):

1. A large variety of different micro-organisms inhabit the rumen.
2. The variation in EAA profiles between major groups of micro-organisms, as well as among the predominant strains within each group, is small to moderate.
3. Protozoa are retained selectively in the rumen and do not contribute to postruminal protein supply in proportion to their contribution to the total microbial biomass.

In contrast to ruminally synthesized microbial protein, there are large differences in the nutritive value of RUP from different protein supplements (Schwab, 1995). First, there are differences in intestinal digestibility, both among and within feedstuffs. Secondly, there also exist large variations in the amount of RUP they contain. Because of these two potential sources of variation, a large difference may exist between the amounts of digestible RUP that one assumes a protein supplement is providing and what actually is being provided. Feed proteins also vary greatly in EAA balance. From the standpoint of formulating diets for a specific pattern of absorbable AA, there seems to be little difference between the EAA composition of a feed protein and the EAA composition of the RUP fraction of the same feed (Schwab, 1995). The EAA profile of the unfermented feed residue is only slightly different from the same feed before exposure to fermentation. For most protein supplements, the contributions of basic EAA to total EAA in RUP were slightly lower than in the same feeds before exposure to ruminal fermentation; in contrast, the branched-chain EAA were slightly higher.

2.3.2 Rumen Undegradable Amino Acids

Various methods have been used to increase the supply of protein and AA to the small intestine, including feeding proteins with high RUP content and chemical or physical treatments which increase the RUP content of a feed. Until recently, productive diets for ruminants have been supplemented with various sources of RUP. Some common sources that used to be used widely include fishmeal, meat and bone meal, feather meal and maize gluten meal. However, since the widely documented cases of BSE internationally, it is now illegal, also in SA, to feed most of these animal by-products to ruminants. Based on AA profiles and rumen degradability, maize and its by-products are relatively good sources of leucine, but are low in Lys. Fishmeal is a good source of Met, but soyabean meal is not. Blood meal is a good source of Lys, but is low in Met. Feather meal is high in branched-chain AA. It is obvious that there is not one perfect source of AA (Rode & Kung, 1996).

Our inability to predict production responses to supplemental RUP are due to a number of factors (Rode & Kung, 1996). The ideal method to measure the RUP content of feedstuffs is *in vivo*, and some labs are not geared for *in situ* analysis either. *In vivo* is more expensive and time consuming as well. The *in situ* technique is most commonly used and was also mainly used to set up the NRC and other data bases. When we alter protein sources, we change RDP as well as RUP content of the diet. This will affect rumen fermentation and consequently, the amount of microbial protein produced. While the differences in RDP content is recognized among feedstuffs, the extreme within-feedstuff variability is seldom considered. In addition, dietary factors that affect microbial access

to the feed (e.g. feed particle size) and rumen environment (e.g. turnover rate, pH, and proteolytic activity) will alter the RUP content of feedstuffs. The effect of DMI and outflow rate on the RUP content of a diet is at least accommodated for in the NRC and CPM-Dairy models. A feedstuff, therefore, do not have a standard RUP value.

Heat treatment has been used to decrease ruminal degradation of proteins and AA. Heating causes carbonyl groups of sugars to combine with free amino groups of proteins during the Maillard reaction. Amino acids also forms peptide links with asparagine and glutamine. The resulting peptide linkages from heating are more resistant to enzymatic hydrolysis. Oil seed protein sources are the most economical to treat with heat. Roasting and extrusion is popular methods to increase the RUP content of soybeans. However, some precautions must be taken when heat-treating proteins, as excessive heat can cause EAA such as Lys, Met and cysteine (Cys) to be extensively damaged (Kung & Rode, 1996).

Increasing the amount of rumen RUP has not always increased the amount, or altered the quality of, AA reaching the small intestine. In some instances microbial protein production has decreased when RUP increased, probably because of a reduction in diet ruminal fermentability (Ferguson *et al.*, 1994). This resulted in an increase in RUP supply but a decrease in microbial protein production, resulting in no net change in total AA flow to the small intestine. No single feed source of RUP provides a balance of EAA that matches the EAA profile of milk. In addition, many feeds with high RUP values are low in one or more EAA. As a result, a deficiency of one AA could be exacerbated by feeding

a RUP source low in that particular AA (Rode & Kung, 1996). Combinations of several RUP that are complementary to each other could help overcome this problem.

When formulating diets, our first goal should be to select dietary ingredients that would maximise MCP. Microbial protein has an excellent profile of AA and the Lys and Met content closely matches that found in milk protein. Thus, feeding a balance of readily fermentable carbohydrate sources with highly digestible NDF sources should be a first priority in order to maximise microbial protein synthesis. It is also important to feed sufficient RDP to ensure the rumen fermentable carbohydrate is effectively transformed into microbial protein. Rumen degradable protein should represent at least 10.5% of DM, and microbial protein should represent at least 50% of MAA supply (Schwab *et al.,* 2003). The remaining MAA will have to come from RUP sources. Usually all RUP sources have lower concentrations of Lys or Met and often both, compared to milk protein. The successful application of balancing for AA lays in careful selection of raw materials that compliment each other in terms of Lys and Met.

Blood meal has the greatest potential to elevate Lys levels due to its high CP, RUP and Lys content. However, in most countries, like SA, animal derived by-products have now been banned from use in animal feed. Only monogastric blood meal can still be used for ruminant feeding in SA and some other countries, but is expensive and not readily available. Fishmeal remains the only other commonly used source that, although not as high in Lys as blood meal, is richer in Met and provides a balanced source of both AA. There are, however, serious consistency problems with this by-product as well as a very

volatile market regarding availability and price (currently more than R9000/ton). Soyabean meal and protected soya products also have higher than average Lys contents and their incorporation in the diet can be useful in meeting target Lys concentrations in MAA (around R4000/ton).

The amount of fishmeal needed to supply 10g of undegradable Met to the small intestine, can be used as a practical example. A nutritionist needs to include 225 to 325g of fishmeal in order to provide the 10g of Met per animal per day. If 15 – 20g of a RPMet can be fed to supply the 10g, it leaves more space in the diet to include other potentially limiting nutrients. Furthermore, the introduction of the fishmeal could over-supply another AA that could reduce the effect of the added Met (Sniffen, 2002).

2.3.3 Rumen Protected Amino Acids

Amino acids can already be added directly to the diets of monogastric animals to overcome nutritional deficiencies. However, free-form AA are rapidly degraded by rumen bacteria and are of little or no practical benefit in alleviating AA deficiencies for ruminants. Rumen protected AA must thus either be modified or protected in some way, in order not to be susceptible to rumen degradation. Furthermore, a balance must be achieved so that AA protected from ruminal degradation are still available for intestinal absorption. In addition, these compounds should be stable in heat when feed are pelleted and in a low pH environment, for example when incorporated into silage-based diets in which the pH can sometimes be as low as 3.6 (Rode & Kung, 1996; Socha *et al.*, 2005).

To supply additional Met and Lys for production of milk and milk protein, various methods and techniques have been developed to protect these AA from microbial degradation, resulting in the RPAA passing to the abomasum and small intestine where they are released and absorbed (Papas *et al.*, 1984; Sloan, 2005; Broderick, 2006b). A considerable effort has been made to develop technologies for supplying Lys and Met in a format that would allow these supplements to escape ruminal degradation without substantially compromising their digestibility in the small intestine. Because the amounts and proportions of AA in duodenal digesta vary when different diets are fed, it is difficult to determine which AA are limiting (Piepenbrink *et al.*, 2004). The AA submodel of the CNCPS has been developed to predict dietary deficiency or excess for growing or lactating cattle (Löest, 2006).

Protein has been, primarily in its component AA form, a primary target for protection technology due to its generally high price and extensive degradation in the rumen. Increases in costs of supplemental protein sources could lead to widespread use of RPAA in dairy cattle diets. Selection of RPAA products by dairy producers should be based on the effectiveness of the product at escaping the rumen intact and releasing absorbable AA in the intestine (Robinson, 1996).

Free AA are not recommended as supplements in ruminant diets because of rapid degradation in the rumen. Thus, chemical alteration or physical protection is required to protect an AA from rumen degradation and to increase the supply of that specific AA to

the duodenum. Ideally, a balance must be achieved so that an AA protected from ruminal degradation is still available for intestinal absorption.

During the 1970s, much of the development of RPAA products was focused on synthetic polymers in which the individual AA, or mixtures of AA, was imbedded (Robinson, 1996, Blum *et al.*, 1999). These efforts were generally successful in that polymers were developed that resisted rumen degradation and dissolved in the mild acidic conditions of the small intestine releasing the AA for intestinal absorption (Papas *et al.*, 1984; Socha *et al.,* 2005). However the high cost of the polymers and health concerns related to polymer residues in body tissues and milk convinced most researchers that this approach was not commercially viable. Recent efforts have focused on developing RP coatings that contain ingredients on the "GRAS" (Generally Accepted As Safe) list of the U.S. Food and Drug Administration (FDA). This have caused most RP research groups to focus on fats, or processed fats, as the RP vehicle (Rossi *et al.*, 2003; Sloan 2005).

A completely different method of improving the supply of AA to the lower gut was reported on by Ohsumi *et al.* (1994). These researchers isolated a Lys-accumulating *Saccharomyces cerevisiae* yeast that, depending on substrates, could accumulate from 4 to 15% of their dry weight as Lys. The majority of Lys was in vacuoles that were stable when incubated with rumen fluid, but immediately released when exposed to pepsin. Thus, feeding this organism could increase the amount of Lys for intestinal absorption.

Metal chelates of AA have been used to improve the bioavailability of minerals. Using the same principle, Zn-Met and Zn-Lys have been used successfully as RPAA sources (Kincaid & Cronrath, 1993). The disadvantage to using Zn-AA chelates is the high level of Zn in the diet. Typical levels of AA supplementation can result in Zn levels being 10 to 20 times above normal.

The work by Rossi *et al.* (2003) measured the *in vivo* ruminal disappearance and the intestinal digestibility of several RPAA and related them to the *in vitro* N solubility data. Eight RPAA were used in the experiment: Lys coated with combinations of long chain fatty acids, triglycerides and calcium soap fatty acids. Both Lys and Met were coated with C16 and C18 Ca-soaps and with C12-C18 hydrogenated fatty acids. Methionine was also coated with ethyl-cellulose as well as with a pH-sensitive polymer. Rumen degradability was assessed with the *in situ* polyester bag technique. The AA intestinal digestibility was also assessed according to the mobile bags technique. Bags were introduced into the duodenum of fistulated cows and recovered from faeces within 19 hours. The *in vitro* AA rumen degradability was predicted according to product solubility in buffer solutions.

Amongst the Met supplements, the lowest rumen degradation was for the pH-sensitive coated product. The data confirm a better resistance towards rumen bacteria attack of the completely esterified cover matrix versus the free fatty acids or Ca-soaps (Rossi *et al.*, 2003). With the same kind of coating, the *in situ* degradation was higher for the Lys compared to the Met products. The estimate of the effective degradability indicates a

lower degradation rate for the Lys products having triglycerides rather than Ca-soap coatings. This is in agreement with the higher AA blood concentration observed when feeding AA coated with a pH-sensitive polymer matrix rather than the ethyl-cellulose coated product (Rossi *et al.*, 2003).

Rossi *et al.* (2003) concluded that the lower rumen degradation was observed when coated with a pH dependent polymer and ethyl-cellulose. However, the latter products reduced the AA availability at the intestinal level. Rumen degradability and intestinal digestibility could be estimated on the basis of nitrogen solubility in buffers. The addition of an enzymatic treatment (pancreatin), after incubating the sample, considerably improved the proposed equations. A shortcoming in this study is that blood AA concentration was not measured.

The second difficulty in utilizing RPAA effectively has proven much more difficult to overcome. Where, when and how much of a nutrient to include in a dairy diet remains a fundamental question to nutritionists. However, in the case of intestinal delivery of RPAA in gram amounts to dairy cows producing 50kg milk or more daily, the questions become even more complex. It is not only necessary to predict intestinally absorbable AA requirements, entailing a detailed understanding of protein and energy metabolism in body tissues, but it is also necessary to predict AA delivery to the intestine from both dietary sources as well as rumen microbes (Socha *et al.*, 2005). Both of these predictions, related to AA delivery to the intestine, rely upon imperfect research procedures and limited amounts of data (Robinson, 1996). The most sophisticated metabolic models of

dairy cows that have been incorporated to diet formulation packages should provide printouts of individual absorbable AA requirements and intestinal delivery to the nearest gram. These models are, in other words, somewhat qualitative (i.e. identifying trends for AA deficiencies among diets rather than specific AA requirements for specific diets).

The effectiveness and profitability of RPAA inclusion to diets for lactating dairy cows in the future will depend on the characteristics of the RPAA product and an ability to predict the intestinally absorbable AA balance. Requirements for successful RPAA products will include (Robinson, 1996):

1. RPAA available by individual AA.
2. RPAA with stated AA levels.
3. RPAA with stated rumen protection levels (and expected changes with differing feeding situations).
4. RPAA with a competitive cost structure.

RPAA are unlike any feed supplement that has previously been widely marketed for dairy cows (Robinson, 1996). Levels of use and desired dietary combinations of specific RPAA will depend upon accurate estimations of the intestinally absorbable AA balance. RPAA will not be a product for all cows and requirements for individual RPAA will vary with diet, milk production and stage of lactation.

Another issue is the use of animal by-products in livestock feeds. RPAA technology is "environmentally friendly" in that it improves the efficiency of protein utilization for dairy cows. Cows are able to produce the same or more milk while being fed lower quality protein feeds.

The latest diet formulation packages, as discussed earlier, guide dairy producers to when and how much of the different RPAA products should be used in diets and has become an important part in the effective use of these products. It is necessary to assume that RPAA products will be supported by a sophisticated diet formulation package to accurately predict situations in which the intestinally absorbable AA balance is deficient or imbalanced for specific AA (Robinson, 1996; Chalupa & Sniffen, 2006). One of the major benefits of correct utilization of effective RPAA is increased yield of milk protein as well as other milk components. In addition, gross efficiency of utilization of dietary N (i.e. milk N output / feed N input) is often increased. Thus, RPAA have the general potential to alleviate specific AA deficiencies at the intestine in order to:

1. Allow greater output of milk and/or milk components.
2. Allow more efficient utilization of dietary N for milk protein synthesis (Robinson, 1996).

In its first role, RPAA is supplementing AA from feedstuffs which escape the rumen undegraded. Thus the cost of the supplemental proteins, or rather the replacement cost of protein by RPAA, is critical to their potential use. In its second role, RPAA may have little effect on total output of milk or milk components, but may improve the efficiency of

utilization of dietary N, particularly where total dietary N levels are decreased. In these situations, RPAA can be utilized to keep the limiting intestinally absorbable AA levels constant (Robinson, 1996).

The most obvious role for RPAA is as a substitute for RUP in dairy diets. For example, to supply one gram of Lys to the small intestine, 86g of soyabean meal would have to be fed. To supply a similar quantity of Met would require 649g of soyabean meal (Rode & Kung, 1996). Alternatively, smaller quantities of blood meal or fish meal could be used to supply the necessary AA. Additionally, when large amounts of protein sources are supplied and thus also acts as a source of energy, the N component of the protein is converted to urea in the liver. This process requires additional energy that could have a significant negative impact on the cow. For example, a cow consuming the 86g of soyabean meal, instead of close to 1g of RPLys, will require additional metabolisable energy to convert the excess nitrogen into urea. Furthermore, providing this amount of energy would require additional feed supply in the diet (Rode & Kung, 1996).

The major factors influencing the overall use of RPAA will be the cost of supplemental proteins and the groups that bear the environmental costs of disposing of waste N. Robinson (1996) discussed the practical application of RPAA under the following scenarios:

2.3.3.1 When supplemental protein costs are low and environmental costs are not taken into account by dairy producers:

In general, dietary protein is overfed to maximise milk output. Under this scenario the use of RPAA is limited to a few situations. One example is where dietary proteins are mainly from the same sources (e.g. high in low Lys maize proteins). Another situation might be where dietary soluble and degradable CP levels are too low to support maximum rumen microbial growth. In these two example situations, specific AA deficiencies may occur, even at relatively high dietary CP levels, and can be corrected with RPAA. However, under a low cost scenario for supplemental protein it would most likely be more cost-effective to change the supplemented protein source to one or more that contain high levels of the deficient AA, or simply to feed more dietary protein. It is doubtful, however, if this scenario would exist again, especially with the rising cost of food and feed that we experience these days.

2.3.3.2 When supplemental protein cost are high and environmental costs are taken into account by dairy producers:

The objective is to keep the overall use of supplemental dietary protein low but to maximise microbial yield with relatively low cost, highly soluble proteins as well as non-protein nitrogen (NPN). The challenge will be to supplement the AA profile of rumen escape proteins of both dietary and microbial origin to optimize the intestinal AA profile for maximum milk and milk component production. Opportunities for use of RPAA will increase. This is the situation that currently exists in SA, as in many parts of the world.

2.3.3.3 When supplemental protein costs are low or high, but environmental costs are important to the dairy producers:

The overall objective under this scenario is to reduce the environmental cost of disposal of excreted dietary N. The efficiency of utilization of AA absorbed from the intestine will have to be maximized. To achieve this goal it will be necessary to provide soluble and degradable proteins in the diet at levels below those required to maximise rumen microbial growth. This will increase the efficiency of utilization of dietary protein and NPN by the microbes, thereby reducing irreversible losses from the rumen. However, this will also reduce rumen microbial escape increasing reliance upon supplementary protein sources which escape the rumen undegraded. The total level and AA profile, of dietary rumen escape proteins must be designed to meet, but not exceed, total absorbable CP needs and optimize its AA profile. It will be virtually impossible to achieve this without the use of RPAA. Thus the use of RPAA will be high, assuming RPAA costs are competitive. The use of RPAA to optimize the AA profile of the minimum required absorbable protein delivery will be the most attractive option.

Various analogs of AA have been tested for resistance to ruminal degradation. One of the more tested AA derivatives is the DL-hydroxy Analog of Met (MHA) (Kung & Rode, 1996). Methionine hydroxyl analog is used widely in the poultry and swine industry as a substitute for Met. One of the most widely known MHA products is Rhodimet AT88™ (Adisseo Animal Nutrition). It is clear that substantial amounts of MHA are degraded in the rumen and that MHA can substitute for Met as either a substrate or stimulant for bacterial growth (Volden *et al.*, 1998). Test results have been variable, with milk fat

percentage increases being the most consistent, but also occasional improvements in milk production (Schwab, 1998). Ruminal effects have included changes in bacteria and protozoa populations and ruminal fermentation patterns. The increased acetate/propionate ratios may even lead to increased fiber digestion due to the fermentation shift towards greater acetate production. *In vitro* experiments indicate that MHA are more resistant to microbial degradation than Met, but similar to Met in stimulating cellulose and glucose degradation and bacterial protein synthesis (Schwab, 1998). Early work of Salsbury *et al.* (1971) suggested that MHA provides Met, rather than just a carbon skeleton, to the ruminal bacteria. Although there might be other modes of action, it is speculated that the stimulatory effects of MHA as well as Met on protozoal growth is as a methyl donor for a number of reactions, including choline synthesis (Schwab, 1998). MHA is also indicated as a preferred source of sulfur (S) for ruminal microorganisms and leading to increased microbial lipid synthesis (Sloan *et al.*, 2000).

Another commercially available MHA is Alimet® (Novus International, Inc). In the chemical structure of Alimet®, the amine group is substituted for a hydroxyl group. This leads to it being available 40% post-ruminally, while some absorption occurs across the rumen wall as well (Patterson & Kung, 1988).

Amino acid mineral chelates have also been used to prevent AA from being degraded in the rumen. These chelates contain about 20-25% AA. Zn-Met complexes were not degraded to any substantial extent in the rumen. Addition of Zn-Met and Zn-Lys significantly increased milk production in cows fed a diet based on maize, soyabean

meal, lucerne hay and grass silage. Fat has been used as a coating material to protect AA but the total proportion of AA has usually been only 30% by weight (Kincaid & Cronrath, 1993). However, results in improving milk production have been variable.

A trial was conducted using a source of rumen-inert Met fed to early lactation cows (Crawley & Kilmer, 1993). Prepartum DMI for cows and heifers assigned to postpartum treatments did not differ. Dry matter intake was depressed for cows fed Met during the first week postpartum. There was no apparent treatment effect on DMI, Body Weight (BW) change, Body Condition Score (BCS) change, milk fat percent or milk total solids percentage through 13 weeks postpartum. Since DMI was depressed for cows supplemented with MHA, it was difficult to detect any positive effects on milk production or protein percent in early lactation. Lower DMI during the first week of lactation indicates that MHA may be unpalatable (Crawley & Kilmer, 1993; Berthiaume *et al.,* 2006).

Polymers that are pH sensitive have been used to encapsulate Met and Lys. These RPAA formulations should be inert in the rumen where the pH is relatively high but would release the AA in the abomasum where the pH is 2 or less (Sloan, 2005). Schwab *et al.* (1993) emphasized that optimizing intestinal AA balance is more important to improving milk protein concentration than is the diet CP or quantity of absorbable protein. In lactating dairy cattle, feeding RPAA has consistently increased milk protein concentration (%) which is important in cheese making, but protein yield (kg/day) has not always been significantly increased. In general, feeding RPAA has not improved DMI

and increases in milk production have been limited (Schwab *et al.*, 1993; Leonardi *et al.,* 2003; Berthiaume *et al.,* 2006; Zebeli *et al.,* 2006).

Rode *et al.* (1994) reported that feeding Lys and Met in a ruminally inert coating increased milk production, milk fat and milk protein production. They also reported that the positive effect continued after the RPAA supplement was withdrawn from the diet. This finding may be due to improvement in total milk per lactation due to increasing peak production, or to some other unidentified metabolic effect. However, a more common finding is typical of the data from Armentano *et al.* (1993) who fed cows in early lactation a combination of protected Met and Lys and reported an increase in milk protein percent, but no increase in milk production. It was also found by Harrison *et al.* (2003) that the CP content of the diet could be reduced from 18% to 16% through the use of RPAA. Veira *et al.* (1991) reported that feeding ruminally protected Met and Lys to feedlot steers improved plasma levels of these AA and increased average daily gain by more than 16% without an increase in DMI. However, in general the growth responses of beef cattle fed polymer coated AA have been inconsistent. These inconsistent production responses to RPAA may be due to the fact that several essential AA are often co-limiting. In addition, some AA, like Met, has several metabolic roles other than a precursor for protein synthesis, as discussed earlier.

Commercial products are limited to Met-Plus® (Nisso America, Inc), Mepron® M85 (Degussa Corporation) and Smartamine™ M (Adisseo Animal Nutrition). In all cases, these are ruminally protected Met products. Currently there are no synthetic bypass Lys

sources on the market. An RPLys product, Smartamine™ ML (Adisseo Animal Nutrition), was withdrawn from the market due to instability. Lys is more difficult to protect than Met. Furthermore, Lys-HCl will not serve as a Lys source for dairy cattle, as the rumen microbes destroy the Lys before it can bypass the rumen (McLaughlin *et al.*, 2002).

Met-Plus® is an example of a lipid-protected product. It is a matrix compound that contains 65% DL-Met embedded in a mixture of Ca salts of long-chain fatty acids, lauric acid and butylated hydroxyl-toluene (BHT): BHT is a preservative for the fatty acids. The technology relies on achieving a balance between ruminal protection vs. intestinal release so as to maximise the amount of Met available for intestinal absorption while minimizing losses in the rumen and faeces.

Mepron® M85 is an example of a surface-coated, carbohydrate-protected product. The pellets consist of a core of DL-Met and starch coated with several thin layers of ethylcellulose and stearic acid. The final product contains a minimum of 85% Met. The technology is a combination of coating materials and application that allows for a large payload of Met. Because enzymatic digestion of the ethyl cellulose is minimal, degradation of the product occurs primarily through physical action and abrasion. The result is a product that slowly degrades in the rumen with a slow release of Met in the intestine.

Smartamine™ M is an example of a lipid/pH-sensitive polymer-protected product. It is a surface-coated product that contains a minimum of 75% DL-Met. The small 2mm pellets consist of a core of Met plus ethylcellulose which is covered with a coat of stearic acid containing small droplets of a polymer (2-vinylpyridine-co-styrene). The presence of the copolymer appears to alter the steriochemistry of the stearic acid such that the surface-coating becomes enhanced in its resistance to ruminal degradation. The presence of the copolymer, as a result of its solubilisation at low pH, also allows for a rapid release of the Met in the abomasum.

In many studies, as in the current study, Smartamine™ M has been used as the reference product against which other technologies are measured (Socha *et al.*, 1994; 2005). Smartamine™ M is estimated to provide 600g/kg as fed of Met. Südekum *et al.* (2002) found a blood plasma increase of > 430 µmol/ℓ, which was more pronounced than the rise for M85 and others. These differences in plasma Met concentrations most likely reflect different degrees of protection of Met against ruminal degradation and/or intestinal absorption.

Further developments has been the esterification of hydroxyl-methyl butanoic acid (HMB) with isopropanol (MetaSmart™ – Adisseo Animal Nutrition), this slows the normal rapid degradation of HMB by the rumen microflora and facilitates absorption across the rumen wall. The result is that the isopropyl ester of HMB (MetaSmart™) provides 370g/kg as fed of Met. It may not have the same payload as Smartamine™ M, but has the added advantage of being pelletable, an important trait that is lacking in any

of the other encapsulated RPMet technologies. The role that HMB plays in the rumen is complex and a precise mode of action has not been validated. As the effects of AA formulation are predominantly on milk protein and the effects of HMB are predominantly on milk fat, the two approaches can be employed together in practical feeding programs to enhance milk volume and components.

Blum *et al.* (1999) conducted a study to compare the bioavailability of D,L-Met of two rumen (polymer and fat) protected Met forms (Smartamine™ M, and Mepron® M85). Blood samples were obtained. Smartamine™ feeding caused elevations of S-containing AA (Met, Cys and Taurine) and reductions of Valine (Val) and Isoleucine (Ile). The feeding of Mepron® caused only a rise in Met concentrations. Concentrations of Met, taurine and glutamine were higher when Smartamine™ was fed compared to Mepron®. Concentrations of non-esterified fatty acids were reduced, but those of insulin were increased only by Mepron® feeding. Milk urea concentrations were lower in cows fed Mepron® than in controls, but milk yields, concentrations of fat, protein and lactose and SCC did not significantly change during the experiment. Food intake, BW and BCS were not affected. In conclusion, only Mepron® supplementation influenced non-esterified fatty acids and insulin concentrations. However, the bioavailability of Met from Smartamine™ was greater than of Mepron® and effects on other plasma-free AA were more marked (Blum *et al.*, 1999). The significantly greater rise in plasma Met indicates that the bioavailability of Smartamine™ was markedly greater than that of Mepron®. The difference in the bioavailability of Met was probably the consequence of differences in rumen protection or absorbability of Met in the small intestine (Blum *et al.*, 1999). If a

rule of thumb for a marginal response is used, then 7g of milk protein can be expected for every additional gram of Met (Sloan, 2005). This means that in a diet needing 10g of additional Met, milk protein yield would increase by 70g per cow per day. Typically there should also be a small fat response. Part of the RPMet ingredient cost would be offset by reducing the amounts (2-4%) of other protein sources in the diet to take advantage of improving overall MAA utilization.

Maximizing the microbial protein contribution should be a first priority when balancing a diet for Lys and Met. Although the pure HMB is a negligible source of Met, it has been shown to enhance non ammonia N flows in continuous culture fermenters through improving the efficiency of microbial protein synthesis. In other words, feeding HMB ensures the concentration of Lys in duodenal flows of protein is maximized and also gives a further opportunity to economize the level of protein in the diet. The benefit of incorporating HMB in the diet is mainly observed on milk fat and in certain studies a large effect on milk volume (Rode *et al.*, 1999), but milk protein percentage has seldom been improved.

The economy of using a RPMet product can be very favourable. This is particularly true if the products are used in conjunction with an overall feeding strategy that is clearly aimed at maximizing the efficiency of milk protein production (Schwab & Boucher, 2007). There are two key factors that influence the economics of feeding a RPMet product (Schwab & Ordway, 2001). First and foremost, there must be willingness and confidence of both the producer and the nutritionist to put “science into practice” and to

use the new models that have been developed that predict concentrations of AA in MP. There must be a willingness to "bend" the protein supplements that are fed and to select high-RUP supplements that complement the use of a RPMet product. There must also be a willingness to accept the fact that improving the profile of EAA in RUP, and thus in MP, reduces the need for RUP (Stern *et al.*, 1997). And second, the economics are enhanced considerably if the producer is paid for milk protein. The cost of RPMet products should not be the determining factor as their cost to deliver a gram of MP-Met is considerably less than high-RUP supplements.

Research into the effect that RPAA may have on the processing quality of milk (Grega *et al.*, 1999) led to some interesting discoveries with possible practical limitations. Supplementing diets with RPMet and RPLys led to improved suitability for cheese-making due to higher casein levels. Increased casein levels will in turn lead to improved cheese yield, especially if casein can be increased above 2.56% (Douglas, 2004). At the same time, however, the supplementation was proven to adversely affect the heat stability of milk.

2.4 Absorption and efficiency of Amino Acid Usage

The factor that is fundamental to achieving the benefit of balancing diets for Met and Lys is improving the efficiency of utilization of MAA. If the dairy cow has an oversupply of all the other AA then the missing links are provided, a whole new milk protein molecule can be synthesised, reducing the surplus of the other AA and improving the efficiency of utilization of MAA. Furthermore, when only relying on MAA to estimate AA requirements, calculations show that actual milk yield falls short of MAA allowable milk in 90% of situations (NRC 2001). In a recent analysis, researchers showed the overall efficiency of utilization of MAA for milk protein secretion to be in the order of 0.64 compared to the NRC (2001) published value of 0.67. MAA utilization was also calculated to be superior to 0.67 when balancing for Met and Lys, both integrated into the formulation approach (Schwab & Ordway, 2004).

It seems to be essential to, as a minimum, pay attention to Lys and Met content of MAA when preferring a factor of 0.67 for the conversion of MAA to milk protein. The studies of Piepenbrink *et al.* (1999) and McLaughlin *et al.* (2002) demonstrated how the correct balance between Lys and Met can improve the efficiency of utilisation of AA. Piepenbrink fed a Met enriched diet and studied the response to increasing supplies of Lys in a dose response manner using a replicated Latin square design. Milk protein secretion increased linearly. The optimum response was an extra 173g of milk protein to increasing daily metabolisable Lys supply by 34g. The efficiency of utilization of MAA

for milk protein synthesis was only 0.53 for the imbalanced diet without any supplemental Lys. Intakes did not change and therefore, at the optimum level of Lys supplementation, the efficiency of utilization of MAA was improved to 0.67. McLaughlin performed a very similar experiment increasing milk protein output by 217g/day through increasing Lys supply by 49.5g.

These results suggest that when MAA is considered as the only entity defining AA supply, there is no estimation of limiting AA and therefore milk performance is likely to be less predictable because of this. Schwab and Ordway (2004) presented an update, which compared MAA, Lys and Met supplies as predictors of milk volume and milk protein yield. MAA supply predicts milk volume adequately and predicts milk protein yield even better. Compared to MAA, Met supply is a better predictor of both milk volume and milk protein yield. However, Lys supply proved to be the best predictor of both milk volume and milk protein yield (Schwab *et al*., 2004). This proves that predictability of milk performance can only be improved by paying attention to at least the first two limiting AA.

By putting emphasis on the Lys : Met ratio during formulation, it is possible to reduce the variation in predicting milk performance. However, by formulating diets only on a MP basis with no consideration for metabolisable Lys and Met, performance will be decreased and be less predictable and milk protein and fat content will not be optimized. Nutritionists should consider integrating a formulation approach to include Lys and Met, allowing diets to be formulated at 16.5-17.5% CP without compromising milk yield and

still improve milk components, instead of continuing the traditional approach resulting in diet formulations of 18% CP or higher (Sloan, 2005).

2.5 Amino Acid Requirements

Three approaches have been used to estimate the EAA requirements of lactating dairy cows: the "factorial" (mathematical), "direct dose-response" and "indirect dose-response" methods. Amino acid requirements can be expressed either in daily amounts (g/d) or on the basis of profiles or patterns. Schwab (1995) prefers the latter because:

1. Profiles can be determined more accurately.
2. It is easier to formulate a diet for a desired pattern of absorbable AA than a given quantity of an AA.
3. The nutritionist is in a better position than the researcher to fine-tune on-farm diets for amounts of RUP and RDP.
4. The approach is consistent with the concept of "ideal protein", as proposed and used in poultry and swine nutrition.

2.5.1 The factorial approach

Scientists from several countries have proposed mathematical models to quantify AA requirements of lactating dairy cows. The CNCPS for evaluating cattle diets and associated AA submodel is the most dynamic of the factorial models. The requirements are expressed on the basis of both daily amounts (g/d) and profiles (each EAA as a percentage of total EAA). Of particular interest is the lack of influence of level of milk production on the "predicted" proportional requirements of most EAA, including Lys and Met (Schwab, 1995, Sniffen & Chalupa, 2004).

2.5.2 The direct dose-response approach

The use of this approach to determine AA requirements of lactating dairy cows is extremely limited and restricted to Lys and Met. Rulquin *et al.* (1990) and Schwab *et al.* (1992) conducted experiments to determine the required contribution of Lys to total EAA in duodenal digesta for maximum synthesis of milk protein. In all six experiments, duodenally cannulated Holstein cows were infused with graded levels of Lys with a constant amount of Met being infused to ensure that Met was not limiting. Estimates for the required content of Lys in total EAA flowing to the small intestine averaged 14.7%. In contrast to the Lys experiments in which milk protein responses plateaued and a requirement could be determined, this was not the case for most of the Met experiments. The infusion of different amounts of Met caused linear increases in milk protein content. It was concluded that Lys needs to constitute about 15.0% of total EAA in duodenal digesta for maximum content and yield of milk protein and that Met needs to constitute about 5.3% of total EAA in duodenal digesta when levels of Lys approximate 15.0% of total EAA.

2.5.3 The indirect dose-response approach

This approach involves 3 steps (Schwab, 1995):

1. Calculating levels of Lys and Met (percentage of total AA or percentage of total EAA) in duodenal digesta for control and treatment groups in experiments in which post-ruminal supplies of Lys, Met, or both were increased (either by intestinal

infusion or by feeding in ruminally protected form) and production responses were measured.

2. Calculating (by extrapolation) “reference production values” in each experiment for fixed levels of Lys and Met in duodenal digesta that are intermediate between the low and high levels as calculated for most of the experiments.
3. Calculating production responses for control and treatment groups relative to the “reference production values”.

There are furthermore some noteworthy observations (Schwab, 1995):

1. There is a better relationship between milk protein content responses and duodenal levels of Lys than with duodenal levels of Met.
2. When intestinal levels of Met were low, increasing intestinal levels of Met decreased content of milk protein.
3. A comparison of the apparent requirements of intestinal Lys and Met with the contributions of Lys and Met to total EAA in feeds and with the calculated levels of Lys and Met in duodenal digesta of high-producing, early lactation cows indicates the difficulty of meeting simultaneously the required contributions of both Lys and Met for maximum milk protein content.
4. Although done independently, it correlates with recommendations of 7.2% (Lys) and 2.4% (Met) expressed as a percentage of EAA in duodenal digesta.

2.6 Responses to Amino Acid supplementation

Four important elements that can have a marked impact on milk production performance are:

1. Metabolisable AA level in the diet.
2. Intestinal digestibility of RUP feed ingredients in the diet.
3. Balancing diets for Lys and Met.
4. Inclusion of MHA for its ruminal action.

The major pathways to enhance the protein available for the support of milk production are:

1. achieve higher feed intakes;
2. provide feed containing higher amounts of protein;
3. achieve optimum rumen fermentation to produce increased amounts of MCP; and
4. supplement with protein sources or RPAA that escape ruminal degradation in amounts higher than conventional feeds.

With the fourth procedure, selection of protein sources can alter the EAA provided at the small intestine because significant differences exist in AA content of feeds (Chandler, 1994).

A summary of production responses of lactating dairy cows in which increased supplies of Lys, Met, or both were fed in ruminally protected form, or infused into the abomasum or duodenum, resulted in the following most common responses and observations (Schwab, 1995; NRC, 2001; Schwab & Ordway, 2001):

1. The sequence of Lys and Met limitation is determined by their relative concentrations in total diet RUP.
2. Content of milk protein is more responsive than milk yield to supplemental Lys and Met, particularly in late lactation cows. It is also clear that milk protein content responses are immediate and that responses remain similar or become greater after peak production. Responses are independent of levels of milk yield or the genetic potential for milk protein content as reflected by breed differences and casein is the milk protein fraction which is most affected and not the whey or NPN fractions. Increases in milk protein production are the most predictable when the resulting predicted supply of the other AA in MP is near or at estimated requirements.
3. Milk protein responses generally are greater when Lys and Met are supplied together rather than when either AA is supplied alone.
4. Milk protein responses to Lys plus Met are greater when levels of either or both in RUP are low rather than high and often greater when intake of CP is high rather than low. Greater responses to limiting AA with higher intakes of CP probably occur because with increasing levels of dietary CP (particularly RUP), AA passage to the small intestine is increased and, up to a point, any “proportional deficiency” of an AA becomes a larger “quantitative deficiency”. This phenomenon will occur with

increasing levels of diet CP until total AA passage is sufficiently high so that the quantitative deficiency becomes less and less.

5. Increasing duodenal concentrations of Lys and Met often increase content of milk protein more than would be expected by increasing diet CP. These observations support the hypothesis that optimization of intestinal AA balance, either by increasing the proportional contribution of microbial protein to total absorbable AA or by improving the balance of AA in RUP, is more important to maximizing milk protein concentration than is content of diet CP or quantity of absorbable protein.
6. Increases in milk yield to supplemental Lys and Met are limited generally to cows in the first two to three months of lactation when the need for absorbable AA, relative to available energy, is the highest; compared to mid or late lactation.
7. Increases in milk protein production to increases in MP of either of the two AA are the most predictable when the amounts of the other AA in MP is near or at estimated requirements.

It is also possible that the balance of nutrients in the diet plays an important role in how efficient a cow synthesizes Met (Bailey, 2000). The balance of Non-Fibre Carbohydrates (NFC), Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) in particular plays an important role in creating a favourable rumen environment for optimum microbial protein production. This allows the cow to more easily synthesize limiting AA from available N in the rumen which, in turn, reduces the amount of supplemental Lys and Met needed (Bailey, 2000).

A Canadian study (Wilks, 2001) demonstrated that high-producing cows fed at NRC requirements for total protein responded to additional Lys and Met, which were protected from rumen degradation. The result was higher milk production as well as increased protein and fat content. It is also indicated that an increase in RUP intake and concomitant increase in metabolisable Lys and Met is production enhancing for mature high producing dairy cows (Struyk *et al.*, 1998). Growing cattle respond to improved Lys and Met nutrition with variable increases in body weight gain, feed efficiency and variable decreases in urinary N excretion (Wessels & Titgemeyer, 1996).

Published research demonstrated that the principles of balancing diets for Met and Lys should also be applied when formulating transition diets to achieve maximum benefit during lactation (Garthwaite *et al.*, 1998). When cows were fed RPLys plus RPMet immediately prior to parturition and for the first 4 weeks of lactation, a reduction in post-calving metabolic disorders were achieved (Schwab, 1995). In the series of experiments conducted by Schwab *et al.* (1992), the need for supplemental Lys was relatively more important than Met in early and peak lactation. By mid-lactation, Lys and Met tended to be co-limiting. This is supported by the variable response observed when RPMet was supplemented alone. Similar studies, where Lys is the sole supplemented AA, are not available. This is probably due to the greater commercial availability of RPMet products compared to RPLys.

It was demonstrated clearly in an experiment by Chapoutot *et al.* (1992) that milk protein percentage is a more sensitive parameter than milk yield when studying AA

supplementation. The authors used a multiple switch-back experiment as a way to evaluate the responses of individual cows to ruminally protected Lys and Met. Of the forty cows in the experiment, 37 responded with increased content of milk protein, 31 with greater protein yield and 16 with more milk. In virtually every study where infused Met or RPMet and Lys were used, milk protein yield and/or milk protein content increased with supplementation with responses ranging from 4 to 15% (Schwab *et al.*, 1992). More importantly, the increases observed in milk protein tend to be in the casein protein fraction, which has significant importance in cheese production.

There are also several reports of increased percentages of milk fat with increased amounts of Met or Met plus Lys in MP (NRC, 2001). As noted in the NRC (2001), these increases have almost always been observed in conjunction with increases in milk protein. Unlike milk protein responses, milk fat responses to improved Met and Lys nutrition have not been predictable.

Experimental data is still limited as to the magnitude of the production responses that one can expect with early lactation cows when the only change that is made is one of more adequate concentrations of Lys, Met or both in MP (Schwab & Ordway, 2001).

Garthwaite *et al.* (1998) summarized 11 experiments on the subject. When supplementation commenced seven to 21 days before calving, the cows responded well to milk yield, milk protein and milk fat during the first 28 to 112 days of lactation. When the data of two experiments in which there was evidence of overfeeding of RPMet were

removed, the average responses to supplemental AA were greater for milk yield, milk protein (both percentage and yield) and milk fat (only fat yield). When AA supplementation commenced zero to 35 days after calving, however, the cows responded with less milk, similar protein and less milk fat during the next 100 days of lactation.

2.6.1 Feed intake and efficiency

The greatest limitation to dairy cow productivity is DM intake. By feeding a relatively small quantity of RPAA, it is possible to eliminate a much larger quantity of protein supplement from the diet (Rode & Kung, 1996). This makes room in the total diet for other ingredients such as forage or concentrates. Having more room in the diet offers producers much more flexibility in diet formulation.

Early lactation is a time of transition for the high-producing dairy cow. Poor feeding and management during early lactation can result in increased metabolic disorders, decreased milk yield and decreased reproductive efficiency. Each 1kg increase in milk yield at peak production can result in 200-225kg more milk produced during the lactation (Crawley & Kilmer, 1993). Peak milk is influenced by the nutrition of the cow in early lactation and stored body tissue reserves. Dry matter intake increases slowly in early lactation and doesn't peak until well after peak milk production has been reached. Therefore, the cow is in a negative nutrient balance early in lactation. Stored body reserves of energy, protein and minerals serve as a source of nutrients while DMI is low (Crawley & Kilmer, 1993). While energy is relatively available from body fat reserves, labile protein reserves are limited. Feeding high levels (above 20%) of CP in the diet to overcome this protein

deficit can be detrimental to the cow. High levels of CP from sources high in soluble protein can result in excess ammonia in the rumen and mildly toxic levels of urea N in the blood and tissues. This condition is known to affect reproductive efficiency. Using protein sources high in RUP will decrease the amount of ammonia produced in the rumen (Crawley & Kilmer, 1993).

In some experiments, responses in milk protein synthesis to supplemental Lys and Met appears to have resulted because of small increases in feed intake, rather than only an increase in efficiency of N utilization (Schwab, 1995). Effects on feed intake are consistent with the widely observed phenomenon that feed intake usually increases as increasing amounts of a limiting nutrient or nutrients are absorbed. Increased DMI contributes significantly to the milk production response sometimes observed with RUP supplementation. In some cases, more than half of the increase observed from feeding RUP can be accounted for by the indirect effect of increased energy supply, rather than the direct effect of additional AA (Rode & Kung, 1996).

2.6.2 Reduction in metabolic disorders

Feeding diets balanced for AA plays a preventative role for certain metabolic disorders through positively influencing energy balance and improving reproductive performance.

Not only is the efficiency of MAA utilization improved when diets are balanced for Lys and Met, but overall feed efficiency is also (Garthwaite *et al.*, 1998). However, increased feed efficiency in itself may not be a good indicator of a "healthy diet" if it is at the

expense of mobilizing energy reserves too rapidly. This could lead to metabolic disorders and delayed or impaired reproduction. Nevertheless, when diets are balanced for Lys and Met due to the improved efficiency of use of MAA, less energy is needed to eliminate surplus AA N as urea; allowing energy to be used more productively. A further reason that could help explain the improvement in feed efficiency, and in particular energy status, may be associated with the other roles of Met in metabolism, rather than simply as a building block for milk protein synthesis.

It has long been recognized that Met have an important role on hepatic metabolism through its capacity as a methyl donor (Bauchart *et al.*, 1998). Methionine plays a key role in assuring the synthesis of apoprotein B, an essential component in the formation of the Very Low Density Lipoprotein (VLDL) complex which is responsible for evacuating triglycerides from the liver to peripheral tissues. It is hypothesized that Met is acting at three different levels to predispose these effects. Firstly, Met is an essential building block for the formation of apoprotein B. Secondly, Met appears to be involved in the gene transcription and/or translation of mRNA for apoprotein B synthesis. Thirdly, Met may also act as a methyl donor to favour lecithin synthesis which is essential for the elaboration of the hydrophilic envelope of hepatic VLDL. The net effect is a reduction in the risks of fat infiltration of the liver which leads to problems such as fatty liver and ketosis. This effect was illustrated in a study by Durand *et al.* (1992) in which ketosis was controlled.

Methionine and its hydroxy-analog have been used to increase ruminal fibre digestion and alleviate milk fat depression (McCracken *et al.*, 1993). Therefore, Met that is not fully protected from ruminal degradation may contribute to increased milk fat synthesis. Methionine is also used by the body in fat metabolism and synthesis. The response to AA supplementation, in particular Met, can be affected by stage of lactation, body condition, and diet. It is therefore often difficult to predict responses from supplementing a nutrient that has many metabolic roles (Rode & Kung, 1996).

Recently, Sloan (2005) conducted two early lactation studies (four to six weeks post partum) with Holstein cows. Cows were prepared to be over-conditioned at calving and then fed an energy restricted diet early in lactation. Half the cows were fed supplementary Lys and or Met. The supplemented cows improved performance by an extra 2.5kg of milk and an increase of 2.5g/kg in milk protein content (Sloan, 2005). In the second trial the milk performance improvements were also associated with a large reduction in circulating ketone-body levels in the second week of lactation, confirming that enhancing the supply of Met and Lys can help reduce metabolic disorders (Sloan, 2005).

2.6.3 Improved reproduction

It is widely recognized that any diet manipulation that can contribute to minimising metabolic disorders and improve energy status of cows in early lactation should also have the potential to positively influence reproductive parameters (Santos, 2005). Robert & Williams (1997) observed an improved uterine involution (percentage of animals whose uterus has regressed to normal size at 45 days post-calving). This was associated with a

reduced number of inseminations needed for conception. They were able to show that the cows receiving a diet balanced for Lys and Met had higher progesterone levels than control animals, leading to successful ovulation (Robert *et al.*, 1997). This is considered to ensure a strong ovulation. Also during five days after insemination, progesterone levels were higher which is often regarded as a positive factor for successful implantation of the embryo. Thiaucourt (1996) demonstrated in field trials in France (53 farms, 2000 cows) that feeding a diet formulated to be rich in Lys and Met, improved timing to first insemination and calving interval by five days.

Another pathway, through which diet formulation for AA is able to positively influence reproductive function, is by facilitating a reduction in high circulating levels of blood urea through the lowering of dietary CP. There is a generally accepted negative association between plasma, serum and milk urea N and conception rates in high producing lactating cows (Ferguson *et al.,* 1998; Santos 2005). It was found that by overfeeding RUP and RDP in the diet, uterine pH was reduced on day seven of the oestrous cycle of heifers and in the case of overfeeding RDP this was associated with a much lower conception rate.

2.6.4 Role in immune response

In dairy cows, there is some indirect evidence that balancing diets for Lys and Met may be positively impacting the immune system (Sloan, 2005). In the field study of Thiaucourt (1996), the expected improvements in milk protein and improved milk production in early lactation were observed when feeding diets balanced for Lys and Met.

They found somatic cell count (SCC) was reduced by 50,000/ml and speculated that a number of factors could have contributed to this phenomenon – the general immune response is improved if animals have an improved energy status. Furthermore, the extra supply of Met increases circulating taurine levels thought to be important in maintenance of the stability of cell membranes and in anti-oxidant reactions. The synthesis of the keratin ring, a protein rich in Cys, at the extremity of the teat duct may also be improved; enhancing the protection against intra-mammary infection.

At the time, there were no RPLys products commercially available (Chalupa & Sniffen, 2006). A number of companies, however, are actively conducting research on the development of a RPLys product. One such a company, S.A. Bioproducts, developed a RPLys product. The experimental evaluation of this product is described in the next chapter. Apart from the fact that responses to RPLys were not as conclusive as with RPMet (Broderick, 2006), the physical chemical properties of Lys is such that current rumen protection technology is not effective enough. Nevertheless, the animal feed industry eagerly awaits a RPLys product (Tylutki, T; personal communication).

CHAPTER 3 - MATERIALS AND METHODS

3.1 Introduction

All new feed additives needs to be evaluated *in vivo* in order to determine production responses and calculate a potential cost : benefit ratio. The purpose of this study was to evaluate a liquid RPLys product in a lactation study with Holstein cows. The product was developed and supplied by S.A. Bioproducts (1 Dickens Road, Umbogintwini, KwaZulu-Natal, South Africa).

3.2 Location

The study was conducted in South Africa, at the Experimental Farm of the University of Pretoria in Hatfield, Gauteng Province; coordinates 25°45'08" S, 28°15'20"E.

3.3 Animals and experimental design

Thirty high producing multiparous Holstein cows in their second to fourth lactation were used in a randomised complete block design to compare a Lys deficient diet (LYS-), which was sufficient in Met, to the same diet but supplemented with a liquid RPLys product (LYS+). All prepartum and postpartum animal care was consistent with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (1999). The protocol was furthermore approved by the Animal Use and Care Ethics Committee of the University of Pretoria.

Although the experimental period was only 120 days post partum, the duration of the experiment from the time the first cow was assigned to treatment until the last cow completed the experiment was 351 days. The reason for this is that cows were not synchronised to calf within a short period of time, but entered the trial as they calved throughout the year.

3.4 Experimental diets, parameters measured and sample analysis

Cows were moved into a transition group 21 days before calving to adapt the rumen to the post partum production diet. During this period cows were fed 4kg of the Lys deficient diet plus *Eragrostis curvula* hay, *ad lib*. After calving cows were continued to be fed the Lys deficient diet (control) for the first three weeks and were then blocked according to the average production from day 19-21. This approach, however, according to Chalupa *et al.* (1997), misses an important stage of the lactation cycle. Parameters such as lactation number, BCS and BW were also taken into account during the blocking procedure although production was the primary blocking parameter. Thirty cows were randomly allocated, within block, to one of two treatments. The two experimental diets were fed from day 22 until day 120 postpartum.

The difference between the first and last block was 12.6kg/d. The ideal is to have this as small as possible in order to minimize variation. However, most research herds have limited numbers and it is always necessary to find a compromise between numbers of animals and amount of variation and time available to complete the study.

The two experimental total mixed rations (TMR) were formulated using the CPM-Dairy Model (Cornell-Penn-Minor, Cornell University, Ithaca, NY, USA) as shown in Table 1. The lucerne and maize based diet contained 39.2% roughage and the CP in the diets was formulated to be 17%. Although the non-lactating pregnant mature cow has a CP

requirement of around 10% of the ration DM, to meet the requirements of high producing dairy cows (35kg of milk or more) the diet should be between 16 and 18% CP (Nichols, 2004). Rations with CP content below 16% often do not have enough RDP to maximise rumen fermentation; becoming a limiting factor in milk production. However, rations containing more that 19% CP have been shown to decrease reproductive efficiency.

The diets' analyses on a DM basis are shown in Table 1; and the additional CPM prediction parameters as well as AA profile are shown in Tables 2 and 3. The Lys deficient diet was formulated for a RR of 2.4% (Met) and 5.57% (Lys) in MP using CPM-Dairy version 2.0.25 (CPM-Dairy, 2002). Smartamine™ M (Adisseo Animal Nutrition, France SAS) was supplemented to obtain the desirable dietary Met level. The "LYS-" (Lys deficient) diet was then supplemented with a rumen protected Lys (RPLys) product to bring the RR to 7.2 % (Lys) and named the "LYS+" diet. The RPLys supplement was readily consumed by the cows with no cows, refusing to eat the supplement.

Table 1: Ingredients and chemical composition of the two experimental diets (%DM)

	Control diet g/kg DM	RPLys diet g/kg DM
Ingredient		
Lucerne hay (Alfalfa)	313	313
Eragrostis curvula hay, chopped	78	78
Maize meal, finely ground	333	333
Maize gluten feed	78	78
Maize gluten meal	39	39
Cottonseed, whole linted	78	78
Molasses syrup	51	51
Urea	3.9	3.9
Rumen protected fat	12	12
Vitamin / mineral premix[1]	4.9	4.9
Sodium bicarbonate	7.8	7.8
Smartamine™ M	18 g/cow/day	18 g/cow/day
RPLys	-	750 ml/cow/day
Chemical Composition		
Crude protein	170	
Soluble crude protein	328	
Rumen degradable protein	582	
Rumen undegradable protein	425	
Neutral detergent fiber	302	
Fat	53	
ME (MJ / kg DM)	11.0	
Non-fiber carbohydrate	434	
Ca	7.9	
P	3.6	
Mg	2.4	
Na	5.2	
K	13.7	

[1]Contained per ton of feed: 2000mg Co, 3g I, 600mg Se, 170g Zn (inorganic), 50g Zn (organic), 150g Mn, 40g Cu, 500g S, 250g Mg, 20g Fe, 65g Anti-oxidant, 8mil IU Vitamin A, 2.4mil IU Vitamin D3, 40g Vitamin E.

Table 2: AA profile and other CPM prediction parameters for dairy cows consuming the LYS- diet (g/kg DM)

CPM parameter	CPM prediction
NH3 Balance (g/d)	57
Peptide Balance (g/d)	-9
MP Balance (g)	41.0
NP / MP (%)	63.8
MP from Bact (g/d)	1598
MP from RUP (g/d)	1478
prNDF	25.0
Met : % Req	134
Met : RR	2.4
Lys : % Req	93
Lys : RR	5.57
Rulquin Ratios	
Methionine	2.40
Lysine	5.57
Arginine	5.61
Threonine	4.54
Leusine	9.59
Isoleusine	5.05
Valine	5.67
Histidine	2.54
Phenylalanine	5.32
Triptohane	1.30

Table 3: CPM-Dairy Predictions for a cow 120 days in milk, with a BCS of 3.0, BW of 605kg and DMI of 25.5kg (LYS- diet)

Target Milk	45 kg/d
Milk fat	3.7%
Milk CP	3.3%
DMI predicted	24.1 kg/d
ME allow milk	45.6 kg/d
MP allow milk	45.9 kg/d
AA allow milk	40.3 kg/d
1st limiting AA	Lys
MUN predicted	17 mg/dl

Cows were housed in groups of eight and were able to move around freely in a dirt exercise lot of 200m² (25m²/cow). Clean water was available at all times. Cows were fed for *ad lib* consumption using a Calan® headgate system (American Calan Inc., Northwood, NH, USA) for monitoring of individual feed intake. Cows were fed twice daily, in the morning at 06h00 and again in the afternoon at 16h00. Although cows were individually fed, the same diet was fed to all the cows within a group. This was done to ensure that cows would at least consume the same diet in the unlikely event of a cow being able to open more than one gate. Cows were fed enough to ensure feed refusal of at least 5%. Cows were milked three times per day, at 05:00, 12:00 and 19:00 in a 10 point herringbone parlour equipped with a DeLaval Alpro milking system (DeLaval Group, Gustaf de Lavals väg 15, Tumba, Sweden) with automatic identification, milk recording and cluster removal.

The RPLys product was in a liquid form and is described in Table 4. 750 ml of the rumen protected Lys contained 52.13g of metabolisable available Lys, which was the amount necessary to reach the optimal Lys to Met ratio, based on an average DMI of 25.5kg/d, was supplemented to the LYS- diet. The supplemental Lys was dissolved in three liters of water and thoroughly mixed into the ration each day. Only water was added to the rations of cows not receiving the rumen protected Lys. More water was then added to both rations to increase the moisture content of the total mixed diet to 30%.

Table 4: Product information of the liquid rumen protected lysine used to study the productivity of Holstein cows

Description	**Result**	**Comments**
Physical form	-	Liquid
Density	1170 g/l	-
Total Solids	58 % m/m	Contains Lys, protector compound & salts
Percentage total Lys in liquid	30 % m/m	Includes all forms of Lys
Percentage sodium in liquid	4 % m/m	-
Bypass value	66 % m/m	Best estimate of protected Lys from analysis
Hydrolysabilty	30 % m/m	30% of Bypass Lys is hydrolysable at pH 2.4, 37°C, 2 hours

Milk production and feed intake were measured daily; milk samples were taken weekly during the afternoon milking and analysed for fat, protein, lactose, SSC and MUN using the System 4000 Infrared Analyzer (Foss Electric, Hillerod, Denmark).

Butterfat was corrected to "24 hour butterfat" values by using the following formula, as supplied by the SA Holstein Association:

24h Butterfat = [28.6754 + (0.6021 * BF% * 100) + (0.3469 * Prot% * 100) + (0.0552 * minutes between previous milk weight and milk weight 1) – (0.1095 * milk weight 1 * 10)] / 100

Further measurements included BCS, (score 1 to 5, with 0.5 unit intervals, where 1 = very thin and 5 = obese, Wildman *et al.*, 1982) and BW; which were both monitored every second week. Additionally, milk samples were taken on day 50 of each cows' lactation and analysed for milk N fractions (casein, whey and NPN). On these samples the factor 6.38 was used for the conversion of N content to protein.

Samples of the experimental diets were collected weekly and composited by treatment. Feed samples were analysed for OM, CP, EE, Ca and P (AOAC, 2000), NDF and ADF (Van Soest *et al.*, 1991) and NFC were calculated (Hall, 1998). The formula used to calculate NFC (de Ondarza, 2000; Harris 2003) were:

NFC (%DM) = 100 - (%NDF + %CP + %Fat + %Ash)

Samples of orts were taken weekly, pooled within treatment, frozen and analyzed at a later stage for CP and NDF to ensure that no selection of feed ingredients occurred. All feed and orts samples for analysis were ground through a 1mm screen (Arthur H, Thomas, Philadelphia, PA, USA) and analysed for organic matter (OM) by ashing in a furnace at 600°C for 2 hours, CP according to AOAC (2000) procedure 968.06 and NDF

according to Van Soest *et al.* (1991). All feed samples, both fresh and orts, were converted to DM by drying at 60°C for 48 hours. The reasoning behind this being that there was a difference in DM between fresh feed and day old orts, namely 70% DM for fresh and 75% DM for orts, due to drying out of the feed.

3.5 Statistical analyses

Data from repeated measurements were analyzed by a randomized block design using PROC GLM Analysis of Variance (Statistical Analyses System, SAS, 2001) for the average effect over time. An analysis of variance was used to determine the significance of difference between different treatments and blocks. The parameters were tested for statistical significance by Fischer's test (Samuels, 1989). Significance was declared at $P < 0.05$ and tendencies at $P < 0.10$.

The linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where Y_{ij} = variable studied at a specific time

μ = overall mean of the population

T_i = effect of the i^{th} treatment

B_j = effect of the j^{th} block

e_{ij} = error associated with each Y_{ij}

CHAPTER 4 - RESULTS AND DISCUSSION

4.1 Experimental diets

The control diet was formulated to supply sufficient nutrients for a cow 120 days in milk, with a BCS of 3.0, body weight of 605 kg and consuming 25.5kg/day of DM; while producing 45kg/day milk with 37g/kg fat and 32g/kg CP. For this, the CPM-Dairy prediction model was used to estimate the nutrient requirements. In this model the factorial system is used to calculate metabolisable energy, metabolisable protein and metabolisable EAA requirements for growth, pregnancy and milk production (O'Connor *et al.*, 1993; Fox *et al.*, 1992 & 2004; Bell & Bouman, 2006). Amino acid requirements are also calculated using an ideal protein method (Rulquin & Verite, 1993).

The LYS- and LYS+ differed only in Lys content with the control being deficient in Lys. The RPLys product was added to bring the ratio of Lys : Met in the experimental diet to 3.12 : 1, using the theoretical values of post-rumen Lys availability as supplied by the manufacturer. The diet was formulated to obtain a Met content of 2.4% of MAA as recommended by Rulquin *et al.* (1994) and Schwab (1995) and adopted by the NRC (2001). This level is very difficult to obtain with normal feed ingredients and therefore the required level was reached by supplementing with RPMet, in the form of Smartamine™ M. Smartamine™ M has been used in many studies to balance Met content (Wessels & Titgemeyer, 1996; Blum *et al.*, 1999; Grega *et al.*, 1999; Schwab *et*

al., 2004 and others) as it has been proven and generally accepted to be the rumen protected product with the highest efficacy (Schwab & Sloan, 2007), which consistently delivers more than 75% Met to the lower intestine.

Chemical analyses of both feed samples and orts (not shown) indicated that no selective feeding occurred. It has been reported that maximum intake requires feed refusals of between 5 and 15% (Mertens, 1992), and therefore this was maintained throughout the trial. The chemical composition of consumed diets differed little, if any, from the mean chemical composition of the formulated diets (Table 1). This was partially due to the ration being wet enough (moisture content of total mixed diet 30%), as well as the Calan® headgate systems' feed bunks being narrow and deep; therefore any attempt to sorting by the cow just lead to further mixing. Furthermore, the cows were fed twice a day, resulting in less sorting.

4.2 Dry matter intake, feed efficiency, milk production and milk composition

Data on production parameters were analyzed separately for all cows (15 per treatment) and for cows in the ten highest production blocks. The latter will be referred to as the "High Producers". The effects of RPLys on DMI are shown in Table 1.

Mean intake of DM did not differ between treatments over the 120 day experimental period, regardless whether all cows (P=0.75) or the high producing cows (P=0.66) were considered. Intakes were remarkably close to predicted values, namely 25.4kg DM/day for control and 25.8 kg DM/day for RPLys, compared to the formulated target of 25.5kg DM/day. The high producing cows had a slightly higher intake, of 25.6 and 26.3kg DM/day for LYS- and LYS+ respectively. These intakes also followed the expected trend for cows in the early stage of lactation as shown in Fig.1 (NRC, 2001). These average DMI values are in agreement with other studies. In a summary of 33 studies where cows were fed TMR's, the average DMI was 23.0kg/d (Zebeli *et. al.*, 2006). In the present study, none of the feed intakes were significantly different between groups. These results are also in agreement with various studies (Papas *et. al.*, 1984; Robert *et. al.*, 1994; Blum *et. al.*, 1999; Harrison *et. al.*, 2000; Leonardi *et. al.*, 2003; Berthiaume *et. al.*, 2006), who reported no significant differences in DMI from cows fed diets with or without different levels of supplemental RPLys and/or RPMet. Wright & Loerch (1988) also reported no significant DMI effects in steers, in RPMet and RPLys trials.

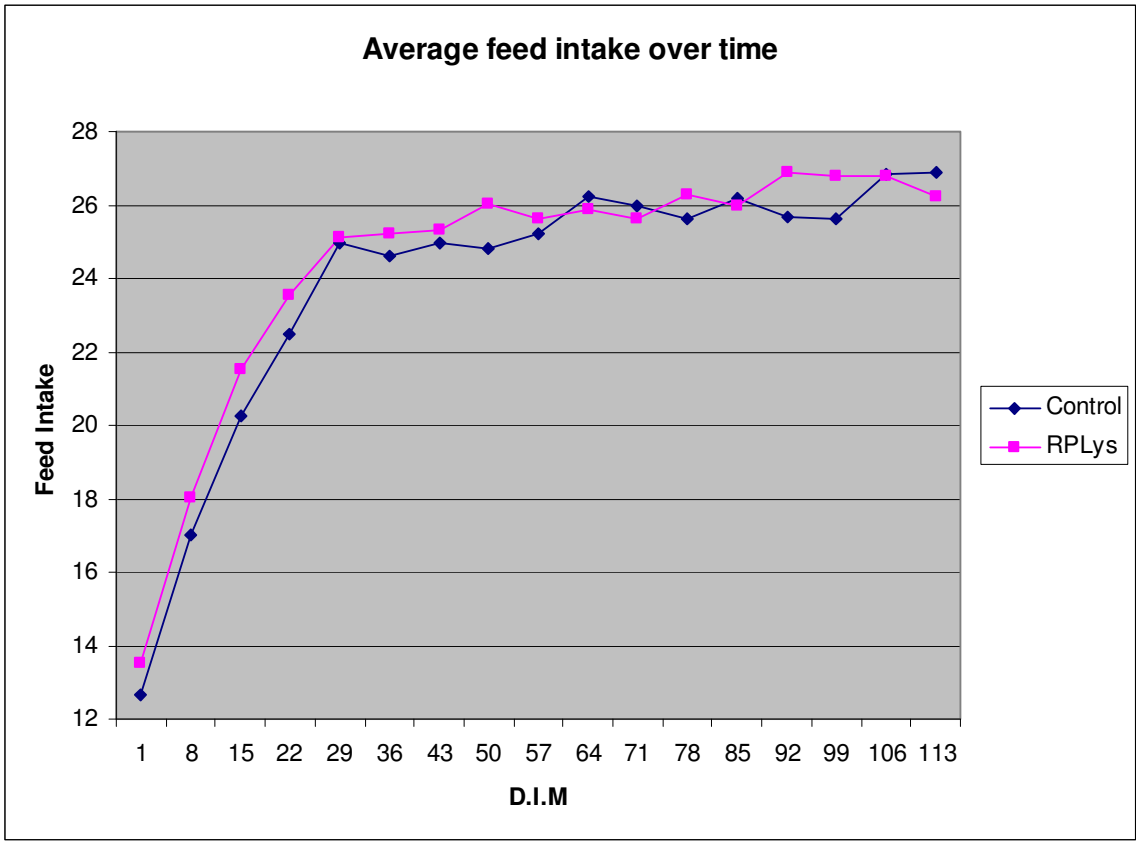
Weekes *et al.* (2006), however, reported a decrease in DMI when an AA mixture, lacking in Lys, were infused into the abomasum, compared to an AA mixture lacking Met. This was, however, observed only for the first three days after infusion; for the total trial period no significant differences were achieved. Similarly, Robinson *et al.* (2000) confirmed a decreased DMI when Lys was lacking and no effect when Met was lacking. Socha *et al.* (1994), however, reported higher DMI for cows receiving RPLys plus RPMet, particularly during peak production. Robinson *et al.* (1995) and Harrison *et al.* (2003) also reported increases in feed intake in response to supplemental Lys and Met. These results, however, might have been due to the widely observed phenomenon that feed intake usually increases as an increasing amount of a limiting nutrient is absorbed (Schwab, 1995). These observations support the use of supplemental RPMet and RPLys, particularly during the critical needs periods of early lactation; the period during which most of these studies have been conducted. Polan *et al.* (1991) also reported that addition of RPMet to a diet sometimes depressed DMI, but the effect was usually reversed when RPLys was combined with RPMet. This reversed effect could support the present study's findings on DMI, in that it remained similar and constant regardless of AA addition.

Table 5: Effect of lysine supplementation on DMI of cows 120 days postpartum

Parameter	Treatment [1]		SEM	*P value*
	Control	RPLys		
Dry Matter Feed Intake (kg/day)				
All cows (n=15)	25.4	25.8	0.80	0.75
High producers (n=10)	25.6	26.3	0.97	0.66

[ab] Row means with different superscripts differ, $P < 0.05$
[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control

Figure 1: Dry matter intake for all cows as influenced by RPLys in the first 120 days of lactation



Mean milk production varied between 40 and 40.2kg/day and was not affected by treatment (P>0.05) (Table 2). Similar levels of milk production have been reported by others for cows consuming TMR's (Uchida *et al.,* 2003; Zebeli *et al.*, 2006). Milk production for the high producing cows (Table 3) varied between 41.9 and 42kg/day; again with no differences (P>0.05). As can be seen in Fig.2, the lactation curve that was achieved in this study followed the normal pattern as expected for early lactation Holstein cows, with cows peaking between four to six weeks post partum (Tekerli *et al.,* 2000; Druet *et al.,* 2003). The literature has reports of inconsistent results to the effect of Met supplementation on milk yield. For example, in a study reported by Koudele *et al.* (1999), where Lys was supplemented, no results on milk production or milk components were obtained either. Bertrand *et al.* (1998) also reported no effect on milk yield, of a supplemented RPMet and RPLys mixture. Rulquin (1992) and Schwab (1995) summarized all experiments in which Lys, Met or both were either infused into the abomasum or duodenum, or fed in ruminally protected form. They concluded that, generally, content of milk protein is more responsive than milk yield to supplemental Lys and Met, particularly in cows after peak lactation. Increases in milk yield, however, is most likely to occur in cows early in lactation, when the need for absorbable AA, relative to energy, is the highest (NRC, 2001; Nichols, 2004). Also, according to findings by Struyk *et al.* (1998), the increase in milk yield was due mainly to an increased DMI. By feeding graded levels of RPMet, Berthiaume *et al.* (2006) were however not able to increase milk production in multiparous cows. However, Robert *et al.* (1994) were able to increase milk production by feeding RPMet in the first six weeks of lactation, but these cows received the RPMet from two weeks before calving. When different combinations

of AA were supplied with continuous abomasal infusion (Weekes *et al.*, 2006), the Lys deficient treatment achieved a significantly higher milk production than the Met deficient treatment, but a similar yield to the completely balanced AA mixture. These cows were, however, milked only twice daily and fed a low protein diet; and therefore achieved milk yields of around 22kg/day. Uchida *et al.* (2003) compared different MHA supplements and cows achieved productions of 45.5kg/d at week four and 53.3kg/d at week eight postpartum. However, according to Chow *et al.* (1990), responses in AA supplementation trials are independent of levels of milk yield or the genetic potential for milk protein content as reflected by breed differences. To be able to compare various lactation trials, the Fat Corrected Milk (FCM) and Energy Corrected Milk (ECM) were also calculated and are discussed later.

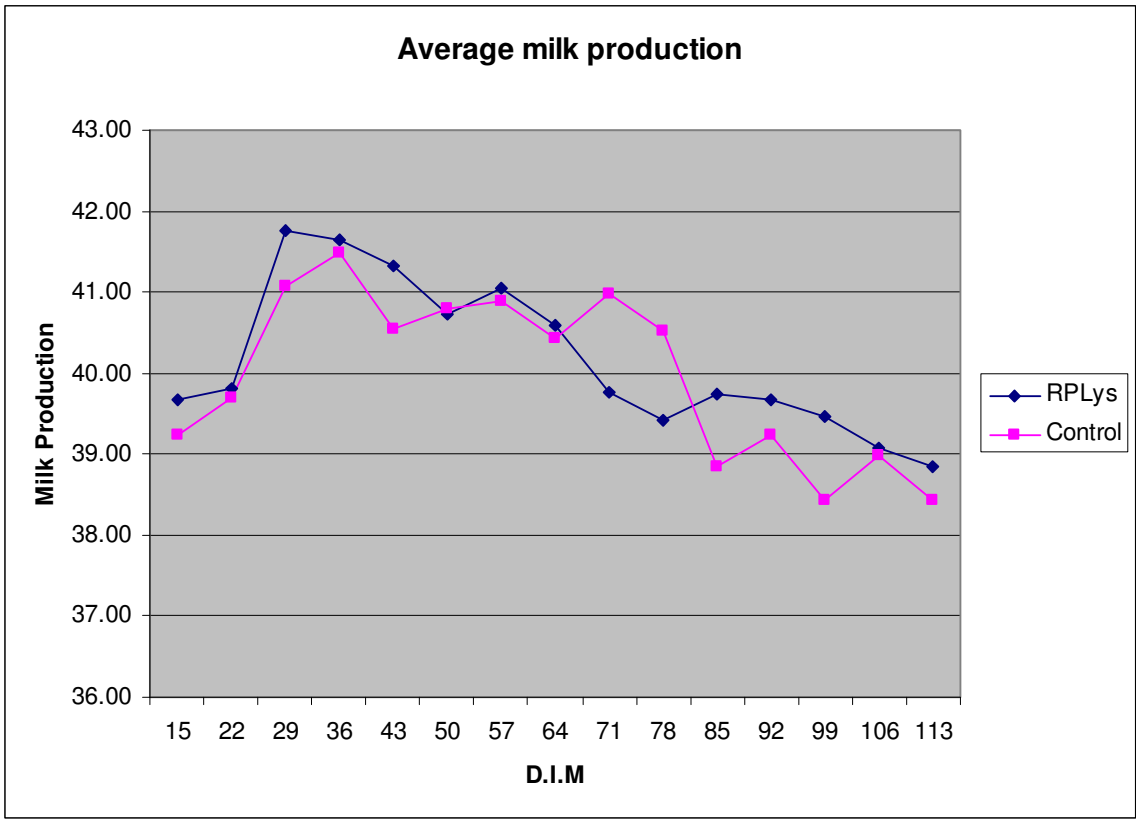
Although published production responses were not always consistent with RPLys supplementation, the majority of reported studies suggest an early lactation production response with RPLys supplementation, especially with such a severely deficient Lys diet as fed in this study. Therefore, milk production and FCM results suggest that the product being tested failed, as no increase in milk production was observed. If this was the case, both treatments were in reality fed a diet lacking Lys, but supplying enough Met allowable milk to achieve productions according to the CPM models' estimate. This estimate was for AA allowed milk of 40.3kg/d (although Met were second limiting) and this was achieved. As balancing diets on the basis of AA will increase mammary synthesis of protein, the type of production responses will vary depending on stage of lactation (Chalupa e*t al.*, 1997). Amino acids seem to increase milk volume if started at

or before calving (Robert *et al.*, 1994; Socha *et al.*, 1994). If delayed until peak production, milk volume increases are small, so the main response to AA is expected to be increased milk protein concentration (Chandler, 1996; Williams, 1996; Erasmus, 1997). It is becoming increasingly clear that production studies aimed at improving intestinal AA balance should be initiated at or before calving (Schwab, 1995). Also, both Gartwaite *et al.* (1998) and Chalupa *et al.* (1999) reported that production responses were greater when RPAA were provided both prior to and after calving.

Also a possibility is that another AA might have been more limiting than Lys. However, given the fair amount of success that has been achieved with the CPM-Dairy in field application and many validation studies, this scenario seems unlikely (Chalupa & Sniffen, 2006).

Milk protein content was on average 2.9% which is acceptable for this stage of lactation, and measurements were compiled during the first 120 Days in Milk (DIM). Similar results for this stage of lactation were reported by Papas *et al.* (1984); Chung *et al.* (2006) and Weekes *et al.* (2006). Normally, the concentration of milk protein is highest in early and late lactation and lowest when production is highest (Wilks, 2005). Provided there is not severe protein under-nutrition, increasing protein level in the diet has only a small and inconsistent effect on milk protein concentration (Roche & Dalley, 1996; Leonardi *et al.*, 2003).

Figure 2: Milk production curve for all cows as influenced by RPLys



However, there is generally a positive effect on protein yield (Schwab, 1995; Bertrand *et al.*, 1998; Misciattelli *et al.*, 2003; Weekes *et al.*, 2006). It is also noteworthy that milk protein content increases are usually immediate and obtained within 3 days (Robinson *et al.*, 1995). Protein source can have an effect through increasing either the quantity or the quality of protein reaching the small intestine of the cow. The underlying principles of increasing milk protein via dietary manipulations are generally to either increase the overall quantity of AA reaching the small intestine or to alter the profile of the AA so that

more of the essential and milk protein-limiting AA are available. It has been shown that adding RPLys and RPMet to increase the protein fraction reaching the small intestine by 7.3% (Lys) and 2.5% (Met) as a percentage of EAA, increases both protein concentration and yield (Rulquin *et al.,* 1995). It has also been shown that supplementing with both AA can give an increase in milk protein composition of 0.8g/l, but supplementing with Met alone has a much lower effect (0.2g/l), if any (Roche & Dalley, 1996). Similarly it has been shown that if mixtures or combinations of AA are not in the correct ratio, or are lacking some EAA, then no change will occur in milk protein (Weekes *et al.*, 2006). Robert *et al.* (1994) reported significant milk protein and casein content responses to supplemental RPMet during the first 6 weeks of lactation. On the other hand, Socha *et al.* (1994), Blum *et al.* (1999) and Berthiaume *et al.* (2006) showed no effect to RPMet on milk protein concentrations in maltiparous cows. The latter is also confirmed by studies of Dinn *et al.* (1998), Koudele *et al.* (1999) and Harrison *et al.* (2000) in response to RPMet and Lys. Milk protein appears to be dramatically reduced when diets provide less than 2.1 – 2.2% Met and 6.0 – 6.5% Lys (Sniffen *et al.,* 2001; NRC, 2001).

In this study, taking into account ration, trial design and the data, the outcome is not in agreement with most published results. Similar to milk production, the lack of any effect of the RPLys supplementation on milk protein suggests that the product did not deliver any additional available Lys to the small intestine. However, overall responses to RPMet have been more consistent than to RPLys (Armentano *et al.*, 1997). The lack of significant milk yield or composition responses might also be due to insufficient uptake of Met and Lys by the mammary gland, but this is relatively unlikely because extraction

by the mammary gland of these AA is marked and fast (Mepham, 1982; Guinard & Rulquin, 1995).

Fat content was not different between treatment groups and fat yield varied between 1.71 and 1.75kg/day (P=0.5). However, in the Bonferroni multiple regression procedure (data not shown), fat yield were significantly different in all cows during week seven (DIM 43 – 49). The Lys treatment produced 1.84kg fat/day vs. 1.65 of the control (P=0.0078). In the same week, the ECM was also significantly different between treatments in all cows; control 42.59kg milk/day vs. 45.56kg for RPLys cows (P=0.0163). Looking at all the results in context, however, this increase in fat yield for only one week is probably biologically insignificant. It is most probably coincidental and not diet related. A few cows probably tested extremely low as that was within the cow's peak production week. Most studies also have shown no significant effect on milk fat content in reaction to RPAA supply (Canale *et. al.*, 1990), although some have shown numerical increases (Piepenbrink *et al.*, 1999; Socha *et al.*, 2005; Berthiaume *et al.*, 2006; Chung *et al.*, 2006; Weekes *et al.*, 2006). Only when Lys and His were lacking, there tended to be a significantly elevated level of fat yield (Weekes *et al.*, 2006) and the fat level was also depressed as soon as the Lys and His levels were corrected. These fat elevations during certain AA imbalances have attracted attention in recent years, but are as yet an unexplained consequence of AA supplementation (Cant *et al.*, 2003). The NRC (2001) summarized two theories regarding the ability of CP to increase milk fat and both theories rely on increased Met availability. However, there has been no effect of milk fat in these and some other studies; and the control diet of the present study can also be

regarded as a Met supplemented ration (due to lack of response of RPLys). Therefore, an increase in milk fat content due to increased dietary protein cannot be explained solely by increasing available Met. However, new data from Socha *et al.* (2005) again found a response to RPMet in milk fat only in higher CP diets compared to lower CP diets, further supporting the NRC (2001).

Milk urea nitrogen (MUN) did not differ between treatments and was around 14mg/dl. Urea is synthesized in the liver as an end product of protein metabolism (Jonker *et al.*, 2002). Scientists tend to differ on the ideal level of MUN, but according to Hutjens (1998) MUN should ideally be between 11 and 18mg/dl. Calberry (2003) sets the range at between 11 and 16mg/dl, and the Milk Recording Scheme of South Africa gives an ideal range of between 9.5 and 18.5mg/dl (Personal communication: Norman Mitchell Innes, ARC Irene). Either way, the average in the present trial of 14mg/dl is right in the middle of the ideal range according to various authors. According to Broderick & Clayton (1997), MUN is closely associated with dietary protein and energy. MUN thus acts as an indication of balanced protein and AA nutrition, or rather; a high MUN value can be an indication of too much RDP (Baker *et al.*, 1995). For example, increasing the dietary protein content by 2.7% significantly increased MUN by 3.8mg/dl (Bach *et al.*, 2000). However, in this case, where the ration has been formulated to be deficient in Lys (RR of 5.57% Lys : 2.4% Met), it indicates that this specific AA ratio (Lys : Met), or quantities, does not appear to have a direct effect on MUN output. Even in the LYS+ ration, where the Lys level has been supplemented with the RPLys product to a perfect RR of 7.2% Lys, the MUN were identical. This is consistent with data of Berthiaume *et al.* (2001).

If the Lys : Met ration is skew, we should expect a response on MUN, so this could also mean that MUN is less sensitive for AA imbalances as to protein shortages or oversupply (DePeters & Cant, 1992; Baker *et al.*, 1995). Baker *et al.* (1995) found that only in an excess dietary CP situation, MUN was significantly elevated. Socha *et al.* (2005) showed an increased efficiency of conversion of feed N to milk N with RPMet and RPMet + Lys supplementation. However, responses tended to be inconsistent across dietary CP levels, with efficiency improving in lower CP diets, compared to higher CP diets. The fact that both the LYS- (AA imbalanced) ration, as well as the LYS+ ration (RR balanced), showed the same effect on MUN, serves as another indicator that the supplemented RPLys product failed.

Feed efficiency for the control group, calculated as milk production divided by DMI, was 1.64kg of milk produced from every kg DM feed consumed; compared to 1.60 for the control (P=0.64). Cows in the high producing blocks receiving the control diet produced 1.71kg milk per kg DMI, compared to the RPLys group's feed efficiency of 1.64 (P=0.58). This correlates well with other local TMR studies using similar diets and cows (Erasmus *et al.*, 2005; Bester *et al.,* 2006, Hagg *et al.,* 2008). Socha *et al.* (2005) however, found an increased efficiency of conversion of DMI to ECM when feeding RPMet and RPMet + Lys. Hutjens (2005) proposed a measurement of feed efficiency that corrects for protein as well as fat, which is more appropriate where the effects on milk protein yield are also important. When this method was applied to seven early lactation milk performance trials from Garthwaite *et al.* (1998), the average improved feed

efficiency was calculated to be +0.08 (Schwab & Sloan, 2007). These calculations were not performed in this study.

Table 6: The effect of RPLys supplementation on milk yield, composition and production efficiency of all cows (n=15)

Parameter	Treatment [1]		SEM	*P value*
	Control	RPLys		
Yield (kg/day)				
Milk	40.0	40.2	0.58	0.83
Fat	1.71	1.75	0.04	0.50
Protein	1.18	1.20	0.02	0.57
3.5% FCM [2]	45.0	45.8	0.83	0.55
ECM [3]	43.3	43.9	0.75	0.54
Milk composition				
Fat %	4.28	4.33	0.07	0.59
Protein %	2.97	2.98	0.03	0.86
Lactose %	4.92	4.96	0.03	0.40
MUN (mg/dl)	13.8	14.3	0.68	0.60
Efficiency				
Feed Efficiency [4]	1.64	1.60	0.06	0.64
3,5% FCM [5]	1.85	1.82	0.07	0.83
ECM [6]	1.77	1.75	0.06	0.82

[ab] Row means with different superscripts differ, $P < 0.05$
[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control
[2] 3.5% Fat corrected milk = (0.4255*milk yield)+(16.425*(%fat/100)*milk yield)
[3] Energy corrected milk = (0.3246*milk yield)+(12.86*fat yield)+(7.04*protein yield)
[4] Feed efficiency = milk yield/kg DMI
[5] 3.5% Fat corrected milk efficiency = (3.5% FCM production/kg DMI)
[6] Energy corrected milk efficiency = (ECM production/kg DMI)

Table 7: The effect of RPLys supplementation on milk yield, composition and production efficiency of high producers (n=10)

Parameters	Treatment [1]		SEM	*P value*
	Control	RPLys		
Production (kg/day)				
Milk	42.0	41.9	0.81	0.94
Fat	1.79	1.84	0.06	0.51
Protein	1.24	1.26	0.02	0.60
3.5% FCM [2]	47.2	48.1	1.16	0.60
ECM [3]	45.3	46.1	1.05	0.59
Milk composition				
Fat %	4.26	4.37	0.10	0.43
Protein %	2.97	3.00	0.03	0.43
Lactose %	4.90	4.95	0.03	0.26
MUN (mg/dl)	13.9	14.0	0.90	0.93
Efficiency				
Feed Efficiency [4]	1.71	1.64	0.08	0.58
3,5% FCM [5]	1.91	1.88	0.10	0.81
ECM [6]	1.84	1.80	0.09	0.80

[ab] Row means with different superscripts differ, $P < 0.05$
[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control
[2] 3.5% Fat corrected milk = (0.4255*milk yield)+(16.425*(%fat/100)*milk yield)
[3] Energy corrected milk = (0.3246*milk yield)+(12.86*fat yield)+(7.04*protein yield)
[4] Feed efficiency = milk yield/kg DMI
[5] 3.5% Fat corrected milk efficiency = (3.5% FCM production/kg DMI)
[6] Energy corrected milk efficiency = (ECM production/kg DMI)

Garthwaite *et al.* (1998) summarized twelve published feeding trials concerning the effects of supplementing diets with metabolisable Lys and Met. Firstly, they reviewed seven trials similar to the present study, commencing immediately post calving or within the first two to three weeks of lactation and continuing to at least 120 days in lactation. In these trials daily milk yield was increased an average of 0.68kg, milk protein yield by 80g

and milk protein percentage increased by 0.16 percentage units. Secondly, they summarised five similar studies where the diets were supplemented with Lys and Met in the steam-up ration as well as for the first third of lactation. In these studies, daily milk yield was increased on average by 2.27kg, milk protein by 112g/d and milk protein percentage increased by 0.09 percentage units. In these five studies, daily milk fat yield was also increased by 115g/d and milk fat percentage by 0.01 percentage units. In all cases, the AA balanced diets had either the same or lower levels of dietary CP than the basal diets. Furthermore, data from Socha *et al.* (2005) proposes that cows fed a lower CP content diet (16%), compared to cows receiving a higher CP diet (18.5%), showed a milk protein and fat response to RPAA early in lactation, versus cows on the high CP diet that responded only during mid-lactation. The summary by Garthwaite *et al.* (1998) not only showed the benefits of enriching diets with Lys and Met on milk performance, but it also showed that the principles of balancing rations for Met and Lys should be applied from the start of the transition period to extract maximum benefit during lactation. Based on this, it would have been better to start the trial two weeks pre-partum and use previous lactation data for the blocking procedure. However, due to limited numbers of animals, it was not possible to decrease variation within blocks sufficiently when blocking on previous lactation milk production alone. By blocking on actual milk production between day 18 and 21, it was possible to reduce variation more accurately.

Sloan *et al.* (1998) used CPM-Dairy to examine responses to Met and Lys in the data set compiled by Garthwaite *et al.* (1998). Daily increases in milk yield of 1.7kg, yield of milk protein of 90g/d and concentration of protein in milk of 0.10% occurred only when

Met in MP was greater than 2.2%, Lys greater than 6.8% and the Lys : Met ration exceeded 3 : 1. Similarly to Sloan, Chalupa *et al.* (1999) used CPM-Dairy to formulate AA enriched fresh cow diets. Methionine in MP was increased from 1.89-2.35% and Lys from 6.38-7.45%. The Lys : Met ratio was 3.2 : 1. Feeding the AA enriched diets increased mammary synthesis of protein in both multiparous and primiparous cows, but because milk yield increased in multiparous cows, the increased mammary synthesis of protein was diluted and concentration of protein in milk was unchanged. Feeding the AA enriched diets did not affect mammary synthesis of fat in either multiparous or primiparous cows. Schwab *et al.* (2003) also examined the impact of increasing concentrations of Met and Lys in MP in six commercial dairies. Lysine was increased by adding blood meal and reducing distiller's grains and Met were increased with Smartamine™ M like in the present study. Milk yield were not measured, but all herds responded with increases in concentrations of protein and fat in milk.

4.3 Body weight and body condition score

Mean Body Weight and BW change of the two groups were not different during the 17 week lactation period, both for the all cow group and for cows in the 10 highest producing blocks (P>0.05). Body Condition Score and BCS change followed the same pattern with the BCS being 2.8 for both groups, as shown in Table 4 & 5. As illustrated in Fig.3, the BW changed as expected for the first 120 days of lactation. These results are similar to studies where RPMet and RPLys has been supplemented (Canale *et al.*, 1990; Socha *et al.*, 2005; Weekes *et al.*, 2006). The cows were in a negative energy balance until around day 36, after which they started to move back to a positive energy balance. This change occurred after the cows were past their peak production, between week four and five.

From the BW and BCS figures, it became clear that firstly, the groups were blocked properly and the allocation to every treatment was homogeneous, effectively eliminating bias. Secondly, that it seems as if the RPLys product did not have any effect on BW or condition changes, neither gaining nor loosing more or less than the control. This was confirmed by Socha *et al.* (2005), Berthiaume *et al.* (2006) and Chung *et al.* (2006) who also found no AA treatment effect on BCS or BW change, although in the latter study, the cows were in late lactation. It should be noted that variable results have been obtained with Met and Lys supplementation in different studies and that some of these differences

can sometimes be attributed to the effect of primiparous vs. multiparous cows. In this study only multiparous cows were used.

Figure 3: Effect of RPLys supplementation on BW change for all cows (n=15)

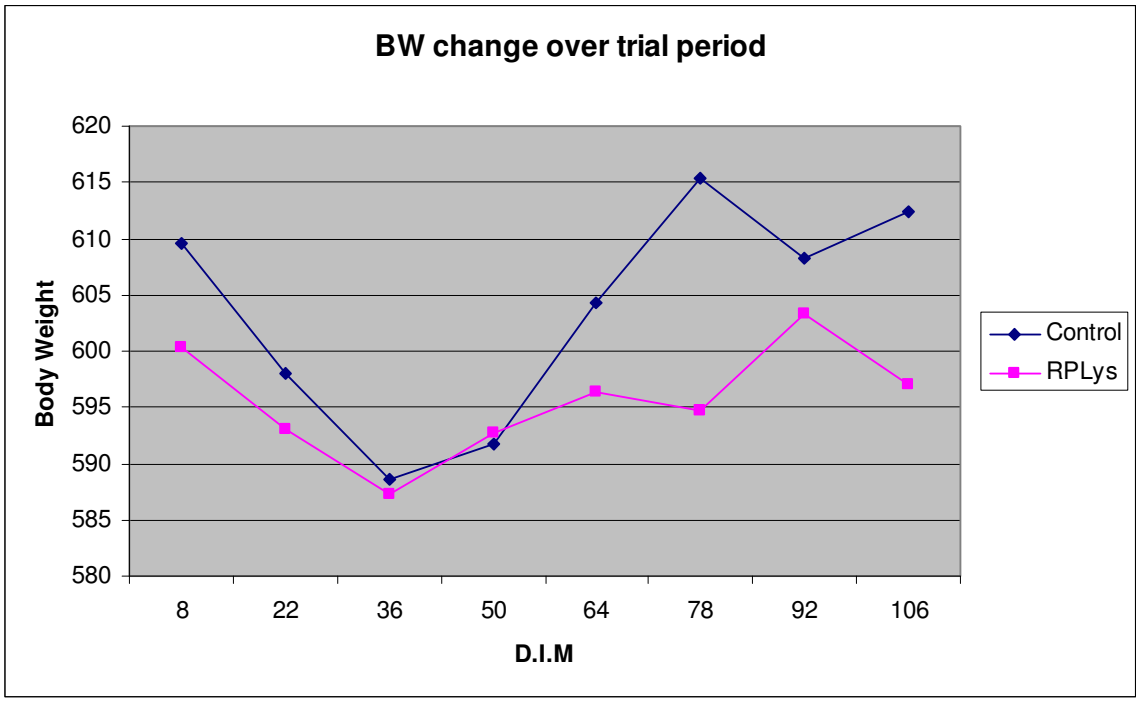


Table 8: Effect of RPLys supplementation on body weight and body condition score of all cows (n=15)

Parameters	Treatment [1]		SEM	*P value*
	Control	RPLys		
Body Weight				
Mean (kg)	602	595	14.60	0.72
Change (kg/120 days)	2.33	-3.33	7.77	0.61
Body condition score				
Mean (units)	2.81	2.81	0.07	0.96
Change (units/120 days)	0.03	0.10	0.13	0.73

[ab] Row means with different superscripts differ, P < 0.05
[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control

Table 9: Effect of RPLys supplementation on body weight and body condition score of high producers (n=10)

Parameters	Treatment [1]		SEM	*P value*
	Control	RPLys		
Body Weight				
Mean (kg)	589	600	19.03	0.67
Change (kg/120 days)	-1.50	-13.00	7.89	0.33
Body condition score				
Mean (units)	2.81	2.74	0.09	0.63
Change (units/120 days)	0.10	0.10	0.16	1.00

[ab] Row means with different superscripts differ, P < 0.05
[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control

4.4 Milk nitrogen fractions

Holstein milk normally contains about 3.2% CP, which is comprised of 78% casein, 17% whey (true proteins) and 5% NPN (Wilks, 2005). Caseins are synthesized from AA, mainly from MP and DP (Williams, 1996). Caseins include αs1-casein, αs2-casein, β-casein and κ-casein; whereas whey consists of primarily α-lactoalbumin and β-lactoalbumin (Yang, 2002). Furthermore, in diets balanced for CP, RDP and RUP; NPN in milk can be divided roughly into 50% urea N and 50% non-urea N (Baker *et al.*, 1995). In this study the milk N fractions, casein, whey, non-casein and NPN were measured. Usually, percentages of whey and casein proteins slowly decline during the first five weeks of lactation (Wilks, 2005). It has been reported that casein is the milk protein fraction which is most affected and not the whey or NPN fractions (Bertrand *et al.*, 1998; Schwab & Ordway, 2001). Robert *et al.* (1994), for example, reported significant milk casein content responses to supplemental RPMet during the first six weeks of lactation. Similarly, Colin-Schoellen *et al.* (1995) demonstrated an increase in casein N in milk, after Lys supplementation; and Berthiaume *et al.* (2006) demonstrated a linear casein percentage increase with increasing levels of RPMet. Chow *et al.* (1990) achieved higher casein percentage when a diet high in added fat was fed, but not with the same diet without fat.

However, casein did not differ significantly in this study (P=0.15 for all cows). This finding is supported by Leonardi *et al.* (2003) and Berthiaume *et al.* (2001). However, a

significant difference was found in whey ($P<0.05$) and non-casein N ($P<0.05$). This was true for all cows but not in the best 10 producer blocks, possibly indicating that the main difference were among lower producing cows. Keep in mind that the lower producers in this study still managed an average production of more than 34kg/day. Whey percentage was, however, not affected by adding AA, in a study by Chow *et al.* (1990), although an increase of whey was observed in a low fat diet. The similar NPN content between treatments is confirmed by no significant differences in MUN (discussed earlier), as measurement of NPN content in milk is a reflection of MUN concentration (DePeters *et al.*, 1992; Roseler *et al.*, 1993; Baker *et al.*, 1995). The bottom line is that RPLys did not affect milk casein concentration, supporting the other production data which points toward product failure.

Table 10: Effect of RPLys supplementation on milk N fractions of all cows (n=15)*

Parameter	Treatment [1]		SEM	*P value*
	Control	RPLys		
N-fractions				
Casein	2.079	2.177	0.05	0.15
Whey	0.631 [a]	0.546 [b]	0.02	0.02
Non-casein	0.807 [a]	0.719 [b]	0.02	0.02
NPN	0.178	0.173	0.00	0.15

[ab] Row means with different superscripts differ, P < 0.05
[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control
* A significant difference in the total group for whey protein and non casein protein was observed.

Table 11: Effect of RPLys supplementation on milk N fractions of high producers (n=10)

Parameters	Treatment [1]		SEM	*P value*
	Control	RPLys		
N-fractions				
Casein	2.094	2.185	0.05	0.24
Whey	0.600	0.558	0.02	0.25
Non-casein	0.772	0.733	0.03	0.30
NPN	0.176	0.176	0.00	1.00

[ab] Row means with different superscripts differ, $P < 0.05$

[1] Rumen protected lysine were supplemented to achieve a Lys:Met ratio in the RPLys diet of 7.2:2.4; and 5.57:2.4 in the control

CHAPTER 5 - CONCLUSIONS

Based upon evaluation of published research, it is proposed that balancing diets on the basis of AA, should increase mammary synthesis of protein, but the type of production response will vary depending upon parity and stage of lactation. Amino acids seem to increase milk volume if started at, or prior to, calving. If delayed until close to or after peak production, like in the present study, milk volume increases are small, so the main response to RPAA is usually increased concentration of protein in milk. Dairy cows in early lactation are sensitive to changes in intestinal AA balance and their lactation performance may be enhanced considerably by optimizing Lys and Met nutrition. The lack of response to RPLys and, for that matter, to RPMet as well, illustrates the importance of characterizing the protein fractions of protein sources used in formulation.

Due to a lack of technical specifications on the product being tested, the reasons for the failure of the product are debatable. If it is then proposed that the product failed, this trial was actually comparing two control rations, both lacking in Lys (as formulated). This once again emphasises the importance of first evaluating products using cannulated animals and using *in situ* or blood ratio techniques before large scale expensive lactation studies are conducted. Although this was suggested to the sponsors of the project, they insisted on a lactation study. Because this was a liquid supplement, the *in situ* technique was unfortunately unsuitable to evaluate this product. For the same reason the mobile bag

technique could not be used to evaluate the product for intestinal digestibility. Although blood sampling could have been done to indicate increased absorption of Lys, this would not have clarified the efficiency of utilisation. Evaluation by means of blood sampling was however not the purpose of the study. There is a possibility that the product was absorbed but poorly metabolised, but the complicated experimental procedures, which would include liver studies, was not considered. Due also to the lack of success of many other companies to rumen protect Lys, the most probable reason for failure was no proper rumen protection. As further evidence, both groups showed similar lactation curves, peak productions, BW and BCS changes. This implies that the effect of the lower and imbalanced RR according to the ideal protein model can not be seen. In other words, the Met in the diets were actually in oversupply. Another diet without any RPAA supplementation, as a negative control, would have been helpful to point out at least any RPMet responses. Ideal RR levels are hard to obtain without single sources of Met and Lys and are therefore on a commercial level not always achieved. Because milk protein levels appears to be dramatically reduced when diets provide less than 2.1-2.2% Met or 6.5-6.8% Lys in MP, these levels are considered minimums. Graphs presented by Rulquin & Verite (1993) suggest that responses of milk protein to Met may be negative if Lys is limiting (i.e Lys/MP < 6.57). Methionine at 150–160% of requirements, depressed DMI and milk yield even when Lys was decreased (Robinson *et al.*, 1996). To avoid potential negative impacts of excess Met, the Lys : Met ratio should then always be 3.1 : 1.

The database used to calibrate the ideal protein model (Rulquin & Verite, 1993) was obtained with cows in mid or late lactation. In most cases milk production was modest. Therefore, responses to increasing proportions of Lys and Met would be expected to be low. It is likely then, that production responses of early and peak lactation cows will be under estimated by the ideal protein model.

Given the higher concentrations of Lys and Met in ruminally synthesized microbial protein than in most feed proteins and the current continuing lack of commercial sources of ruminally protected Lys, the approach for optimizing amounts of Lys and Met in metabolisable protein is to:

1. maximise ruminal synthesis of microbial protein while avoiding the over-feeding of RUP;
2. replace low-Lys protein supplements (e.g. maize gluten meal, feather meal and distiller's grains) with higher-Lys sources (e.g. fish meal, poultry blood meal and soyabean products);
3. feeding vegetable protein products that have been modified (processed) to increase their bypass value; and
4. incorporate a RPMet product in the diet (e.g. Smartamine™ M).

The economics of using ruminally protected AA will vary from farm to farm. It is, however, clear form the literature reviewed, that the economics of using these products (currently referring mostly to RPMet), can be very favourable (Schwab & Ordway, 2001). This is particularly true if the products are used in conjunction with an overall

feeding strategy that is clearly aimed at maximizing the efficiency of milk protein production.

Balancing diets to optimize Lys and Met nutrition is important to maximizing milk and milk protein yields. It appears that establishing relationships between predicted supplies of the most limiting AA in the diet and milk or milk protein yield will allow for more accurate prediction of changes in milk protein production when changes in protein nutrition are made (Schwab & Ordway, 2004). The lack of a rumen protected Lys product and therefore the inability to achieve desired concentrations of Lys in maize based diets, has led to a lot of focussed research in the last few years. Very recently, Balchem Corporation launched a RPLys product "AminoShure™-L". However, no production studies using this product were available yet. Further research and improvement of commercially viable RPLys products should be continued as a matter of urgency.

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